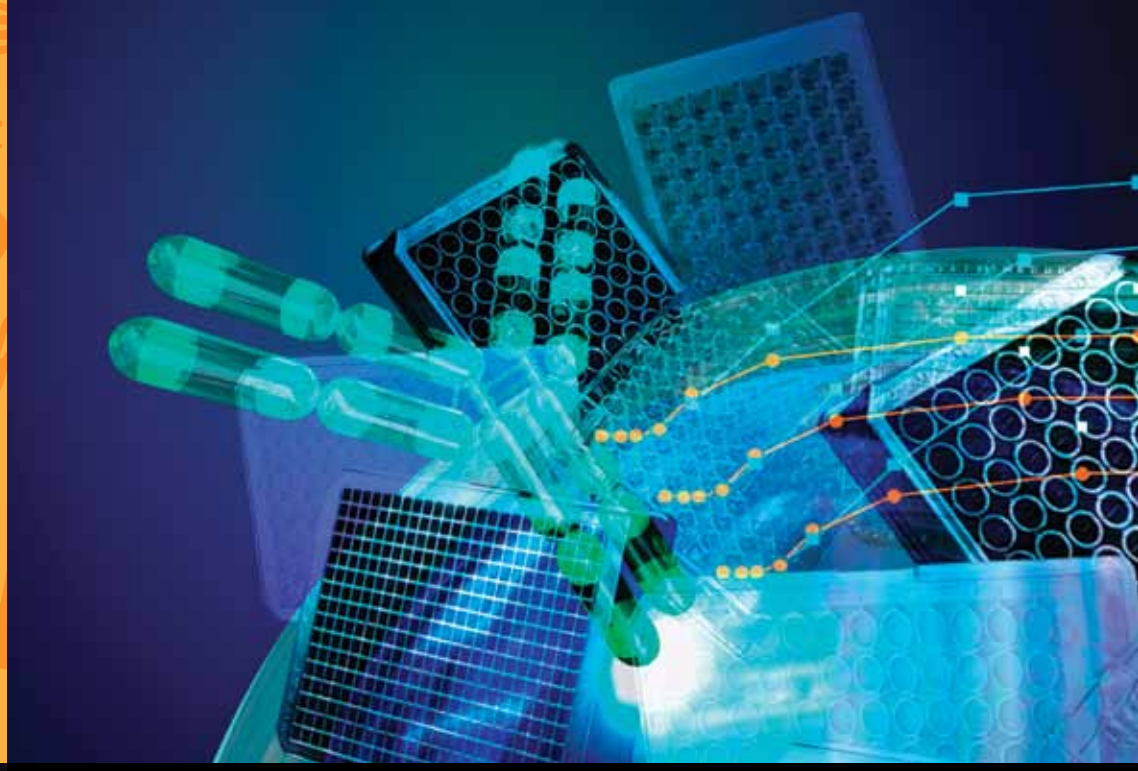

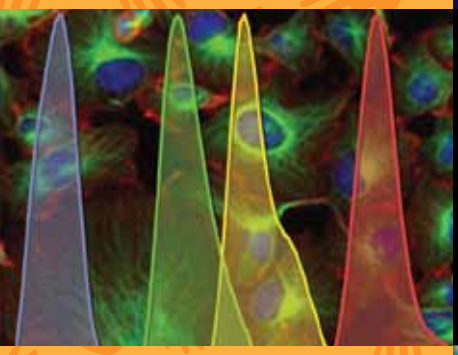

# Thermo Scientific Pierce Assay Development Technical Handbook

Version 2

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

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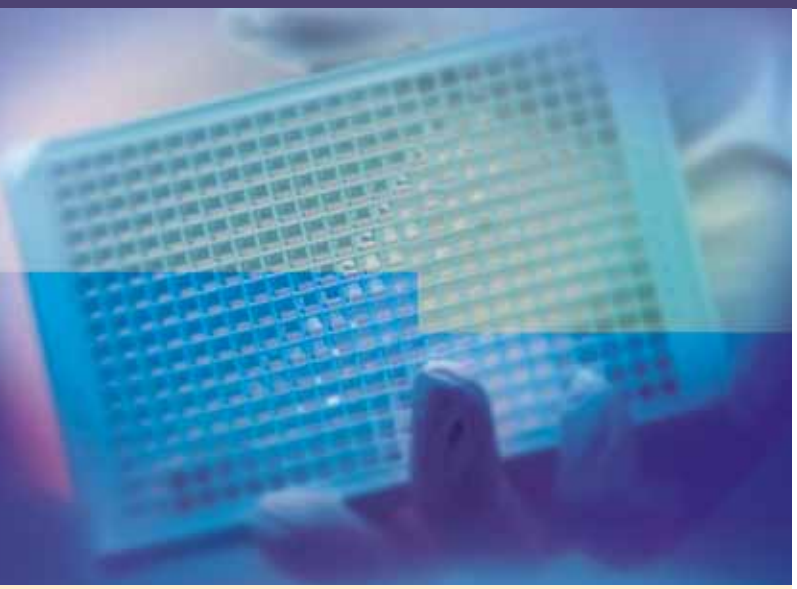
# Introduction



## Enzyme-linked immunosorbent assays (ELISAs)

**ELISAs are designed for detecting and quantitating substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same process.** In an ELISA, an antigen must be immobilized to a solid surface. The antigen is then complexed with an antibody that is linked to an enzyme. Detection is accomplished by incubating this enzyme-complex with a substrate that produces a detectable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

Most commonly, ELISAs are performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform, as first described by Eva Engvall, *et al.*[1] Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from unbound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

A detection enzyme may be linked directly to the primary antibody or introduced through a secondary antibody that recognizes the primary antibody. It may also be linked to a protein such as streptavidin if the primary antibody is biotin-labeled. However the enzyme is incorporated, the most commonly used enzymes are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Other enzymes have been used as well, but they have not gained widespread acceptance because of limited substrate options. These include β-galactosidase, acetylcholinesterase and catalase. A large selection of substrates is available for performing the ELISA with an HRP or AP conjugate. The choice of substrate depends upon the necessary sensitivity level of the detection and the instrumentation available for detection (e.g., spectrophotometer, fluorometer or luminometer).

Similar to an ELISA in general assay design, is the enzyme-linked assay (ELISPOT). The ELISPOT is used to determine how many cells within a suspension are producing a specific cytokine. To perform the ELISPOT, a sample containing live cells is added to wells of a coated microplate. The target secreted from the cells binds to the capture antibody and is then detected by typical ELISA methods.

# ELISA Reagents Summary

## STEP 1A

**Selecting an ELISA plate**
Choose a plate

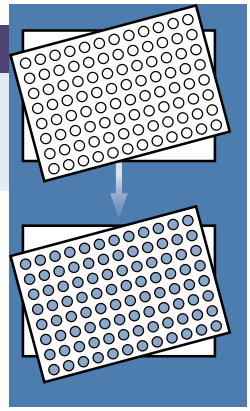
- 96-well Plates (page 9)
- 8-well Strip Plates (page 9)
- Sealing Tape for Plates (page 8)
- Reagent Reservoirs (page 8)
- DNA Coating Solution (page 9)

## STEP 1B

**Pre-coated plates**
Use a pre-coated plate for efficiency and consistency.

- Protein A (page 12)
- Protein G (page 12)
- Protein A/G (page 12)
- Protein L (page 13)
- Secondary Antibodies (page 13)
- NeutrAvidin Biotin-Binding Protein (page 14-15)
- Streptavidin (page 16-17)
- Biotin (page 16)
- Nickel Chelate (page 18)
- Copper Chelate (page 19)
- Glutathione (page 20)
- Maleic Anhydride (page 21)
- Maleimide (page 21)

## STEP 2



**Blocking**
Block unoccupied binding sites.

- Protein-Free Blocking Buffer in PBS and in TBS (page 24)
- Protein-Free T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS or TBS (page 24)
- StartingBlock™ Blocking Buffer in PBS and in TBS (page 23)
- StartingBlock T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS or TBS (page 23)
- SuperBlock® Buffer in PBS and in TBS (page 25)
- SuperBlock T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS or TBS (page 25)
- Casein in PBS and in TBS (page 26)
- BSA in PBS and in TBS (page 26)
- SEA BLOCK Blocking Buffer (page 25)
- BLOTTO in TBS (page 26)
- Nuclease-free Casein (page 27)

## STEP 3

**Add sample and incubate**

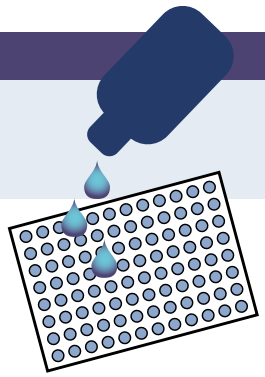
## STEP 4A

**Formulate Wash Buffers**
Choose a buffer.

- Phosphate Buffered Saline (PBS) (page 29)
- Tris Buffered Saline (TBS) (page 29)

## STEP 4B

**Add detergent to buffers**
Reduce nonspecific binding.

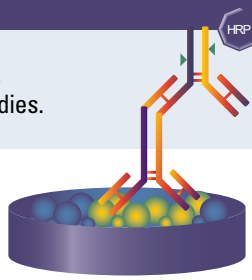


Surfact-Amps® Brand Detergents containing:

- Tween®-20 and Tween-80 (page 28)
- Triton® X-100 and Triton X-114 (page 28)
- Nonidet P-40 (page 28)
- Brij®-35 and Brij-58 (page 28)

## STEP 5

**Primary and Secondary Antibodies**
Incubate the membrane with antibodies.




For a complete list, visit the antibody selection guide on our website (www.thermoscientific.com/pierce) accessible under the Products tab.

**For direct detection methods we offer:**

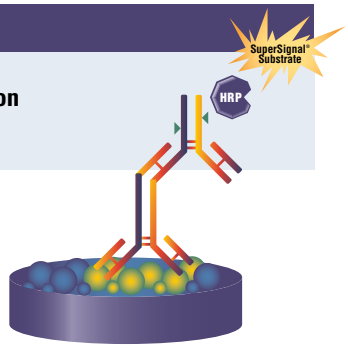
- Monoclonal Antibodies
- Fluorescent Probes and Labeling Kits
- Enzyme Labeling Kits

**For indirect detection methods we offer:**

- Biotinylation Kits
- Protein A, Protein G and Protein L labeled with fluorescein, rhodamine, HRP, AP or biotin
- Avidin, Streptavidin and NeutrAvidin™ Biotin-Binding Protein labeled with fluorescein, rhodamine, HRP or AP
- Secondary antibodies labeled with fluorescein, rhodamine, HRP, AP, biotin or DyLight® Dyes

## STEP 6

**Enzyme Substrates for Detection**
Add the detection reagent.




Chemiluminescent Substrates:

- SuperSignal® ELISA Pico Chemiluminescent Substrate (page 61)
- SuperSignal ELISA Femto Chemiluminescent Substrate (page 62)

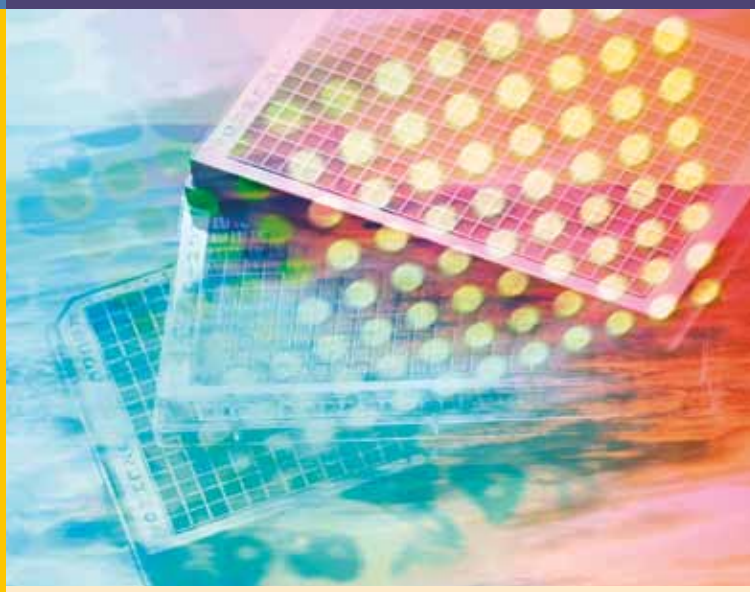
Chemifluorescent Substrate:

- QuantaBlu™ Fluorogenic Peroxidase Substrate (page 63)
- QuantaRed Chemifluorescent HRP Substrate (page 64)

Colorimetric Substrates:

- TMB Substrates (page 66)
- ABTS Substrates (page 65)
- OPD Substrate (page 65)
- PNPP Substrates (page 67)

# Introduction to ELISA



## Typical ELISA Protocol Summary

### Coating antibody or antigen onto the microplate

1. Dilute the protein to be coated to a concentration of 2-10µg/mL in a buffer such as PBS or Carbonate-Bicarbonate and add 100µL of this solution per well.
2. Incubate for 18-20 hours at 4°C or 1 hour at 37°C.
3. Block unoccupied binding sites with a blocking agent (200-300µL/well) such as Thermo Scientific StartingBlock Blocking Buffer.
4. Use plate immediately or store at 4°C with a desiccant.

### Perform assay

5. Add sample to be tested (50-100µL/well) and incubate for 1 or more hours.
6. Wash using PBS with 0.05% Tween-20 or TBS with 0.05% Tween-20.
7. Add enzyme-antibody conjugate (100-200µL/well) diluted in blocking buffer and incubate for 1 hour.
8. Wash again.
9. Add substrate.
10. Detect.

## The Sandwich ELISA

The most commonly used ELISA assay format is the sandwich assay (Figure 1). This type of assay is called a "sandwich" assay because the analyte to be measured is bound between two antibodies – the capture antibody and the detection antibody. The sandwich format is used because it is sensitive and robust.[2] Competitive assays are often used when the antigen is small and has only one epitope, or antibody-binding site.[3] Often the antigen is labeled instead of the antibody. Unlabeled antigen and the labeled antigen compete for binding to the capture antibody and a decrease in signal indicates the presence of the antigen in the sample.

Either monoclonal or polyclonal antibodies may be used as the capture and detection antibodies in sandwich ELISA systems. Monoclonals have an inherent monospecificity toward a single epitope that allows fine detection and quantitation of small differences in antigen. A polyclonal is often used as the capture antibody to pull down as much of the antigen as possible. Then a monoclonal is used as the detecting antibody in the sandwich assay to provide specificity.

An important consideration in designing a sandwich ELISA is that the capture and detection antibodies must recognize two non-overlapping epitopes. When the antigen binds to the capture antibody, the epitope recognized by the detection antibody must not be obscured or altered. Capture and detection antibodies that do not interfere with one another and can bind simultaneously are considered a matched pair and are suitable for developing a sandwich ELISA.

Another design consideration in choosing antibodies is cost. A polyclonal antibody is generally less expensive (approximately five-fold) to produce than a monoclonal. The specificity gained by using monoclonals for both the capture and detecting antibody must be weighed against the cost and time required for producing two monoclonal antibodies. Preparing a "self-sandwich" ELISA assay, in which the same antibody is used for the capture and detection, can limit the dynamic range and sensitivity of the assay.

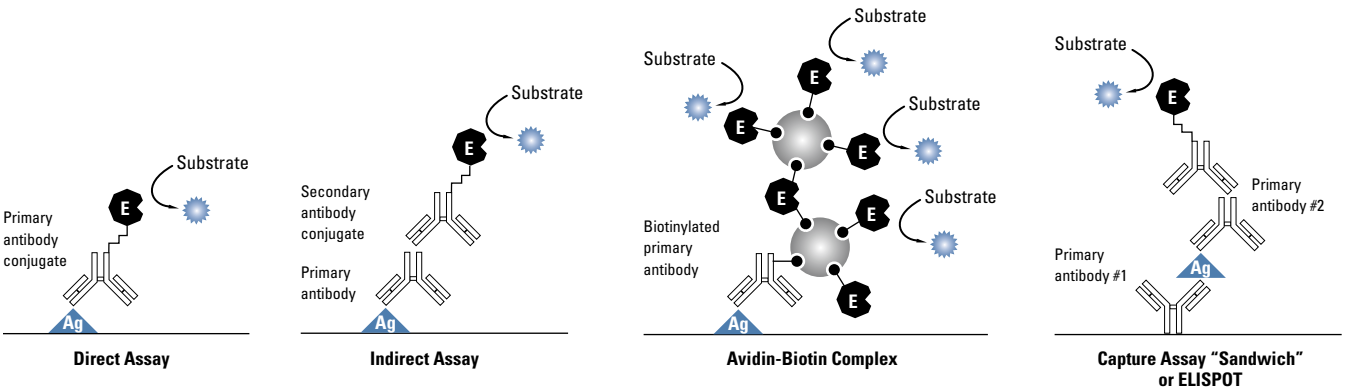



**Figure 1. Immunoassay formats.**

## Direct vs. Indirect Detection Techniques for ELISA

The direct detection method originated in the 1940s when Coons and colleagues labeled antibodies with a fluorescent tag to mark tissue antigens.[4] In this technique, a labeled primary antibody reacts directly with the antigen (Figure 1). Direct detection is not widely used in ELISAs but is quite common for immunohistochemical staining of tissues and cells.

### Advantages of direct detection

- Quick methodology, because only one antibody is used.
- Cross-reactivity of secondary antibody is eliminated.

### Disadvantages of direct detection

- Immunoreactivity of the primary antibody may be reduced as a result of labeling.
- Labeling primary antibodies for each ELISA system is time-consuming and expensive.
- No flexibility in choice of primary antibody label from one experiment to another.
- Minimal signal amplification.

The indirect, two-step method uses a labeled secondary antibody for detection (Figure 1). This was first described by Weller and Coons in 1954 and is still a popular method.[5] First, a primary antibody is incubated with the antigen. This is followed by incubation with a labeled secondary antibody that recognizes the primary antibody. For ELISA it is important that the antibody enzyme conjugate is of high specific activity. This is achieved when the antibody is affinity-purified and the enzyme conjugation chemistry preserves antibody specificity as well as enzyme activity. All of our antibody-enzyme conjugates fulfill these requirements.

### Advantages of indirect detection

- A wide variety of labeled secondary antibodies are commercially available.
- Versatility, because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
- Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification.
- Different visualization markers can be used with the same primary antibody.

### Advantages of direct detection

- Cross-reactivity may occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.

## Developing an ELISA

### Optimizing immunoreagent concentrations and dilutions

The goals in developing an ELISA assay are 1) to achieve the best signal:noise ratio for the sensitivity level desired, 2) to have a robust, reproducible assay for the sample being tested and 3) to be able to measure the antigen over a biologically relevant assay range (dynamic range). Therefore, ideal concentrations of each assay reagent must be established empirically. The signal generated by a sample containing analyte, relative to the signal of the same sample without analyte, is the signal:noise ratio. As the signal:noise ratio increases, the assay becomes better at measuring small amounts of antigen.

Dilution ranges for assay reagents can vary widely, depending upon the detection system used. For example, most ELISA protocols based on enzyme-antibody conjugates using a colorimetric substrate recommend a 1:5,000 dilution (from a 1mg/mL stock concentration) of the conjugate. But this ELISA may work equally well at 1:2000 or 1:20,000 dilution. To establish the optimal dilutions, a checkerboard titration, also called a two-dimensional serial dilution, is performed. A checkerboard titration is a single experiment in which the concentration of two components is varied in a way that will result in a pattern (Figure 2). This method is used to optimize reagent concentrations when performing an indirect ELISA that uses a labeled secondary antibody. For example, the primary antibody is serially diluted across the plate, and the enzyme-labeled secondary antibody is serially diluted down the plate. This design permits analysis of different concentrations of the two reagents in each well to obtain the best signal:noise ratio.

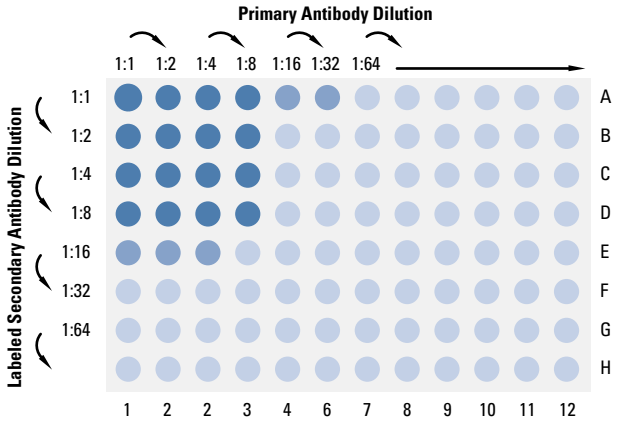



**Figure 2. A checkerboard titration using a precipitating substrate.**

A more complex checkerboard titration for developing an ELISA is illustrated in Figure 3. Here the coating or capture antibody is titered first by using high concentrations of biotinylated detecting antibody and streptavidin-HRP conjugate. Once the signal is within the desired detection range (dynamic range), the checkerboard matrix in Figure 3 can be tested to optimize the concentrations of the biotinylated detecting antibody and streptavidin-conjugate.

**(Coating Antibody at 2mg/mL)**

| Standards | 1:8000 | | 1:16,000 | | 1:32,000 | | 1:8000 | | 1:16,000 | | 1:32,000 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0pg/mL | 0.032 | 0.031 | 0.028 | 0.030 | 0.029 | 0.028 | 0.047 | 0.047 | 0.042 | 0.039 | 0.038 | 0.037 |
| 80pg/mL | 0.225 | 0.219 | 0.208 | 0.207 | 0.167 | 0.175 | 0.402 | 0.402 | 0.363 | 0.369 | 0.287 | 0.294 |
| 400pg/mL | 0.555 | 0.545 | 0.605 | 0.561 | 0.438 | 0.431 | 1.603 | 1.108 | 1.034 | 1.032 | 0.742 | 0.768 |
| 2000pg/mL | 2.870 | 2.620 | 2.494 | 2.476 | 2.037 | 1.770 | **** | **** | **** | **** | 2.709 | 2.866 |
| 0pg/mL | 0.041 | 0.040 | 0.032 | 0.031 | 0.031 | 0.030 | 0.074 | 0.067 | 0.060 | 0.059 | 0.052 | 0.048 |
| 80pg/mL | 0.337 | 0.335 | 0.293 | 0.296 | 0.229 | 0.232 | 0.448 | 0.411 | 0.436 | 0.419 | 0.357 | 0.347 |
| 400pg/mL | 0.831 | 0.886 | 0.838 | 0.829 | 0.604 | 0.613 | 1.466 | 1.348 | 1.243 | 1.255 | 0.891 | 0.884 |
| 2000pg/mL | 3.720 | **** | 3.263 | 3.454 | 2.373 | 2.468 | **** | **** | **** | **** | 3.187 | 3.141 |

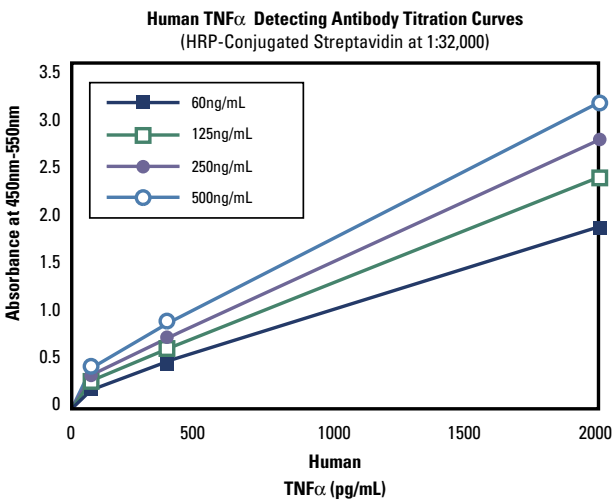
Biotin-Labeled Detecting Antibody Concentration

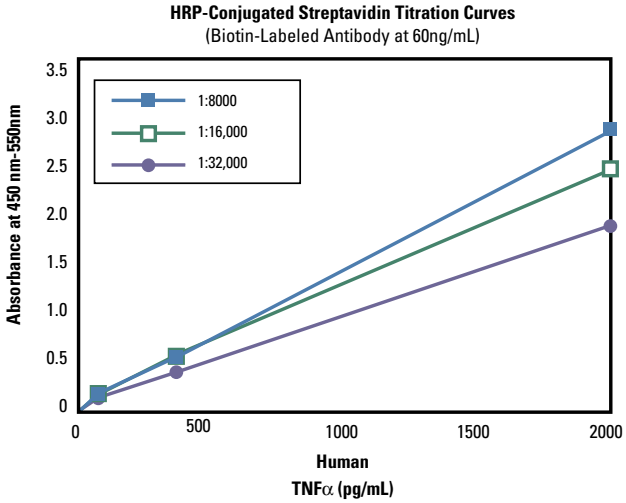
60ng/mL
125ng/mL
250ng/mL
500ng/mL

**HRP-Conjugated Streptavidin Dilution**

**3A.** The plate is divided into four equal quarters for four different detecting antibody concentrations. For each detecting antibody concentration, three different enzyme concentrations are tested on three different levels of standards, plus a zero. Prior to this checkerboard titration, a coating titration was performed to determine the optimal concentration for the coating antibody.

From this checkerboard matrix, the following curves are generated:




**3B.** When dividing the mean O.D. for the 80pg/mL standard by the O.D. for the zero standard, 250ng/mL produced the greatest signal:noise ratio, suggesting the antibody is on a plateau. Using this detecting antibody at 250ng/mL would provide optimal sensitivity, assay reproducibility and sample recovery.

**3C.** The standard curve is within the desired O.D. range at the 1:32,000 dilution of HRP-conjugated streptavidin. For increased sensitivity with a smaller assay range, a higher concentration of HRP-conjugated streptavidin may be selected. Poly-HRP streptavidin may also be used.

**Figure 3. Checkerboard matrix: example of human TNFα detecting antibody and enzyme titration.**

**References**


1. Engvall, E. and Perlmann, P.O. (1971). *Immunochemistry* **8**, 871-875.
2. Palomäki, P. (1991). *J. Immunol. Method* **145**, 55-63.
3. Rao, P.N. and Taraporewala, I.B. (1992). *Steroids* **57**, 154-161.
4. Coons, A.A., *et al.* (1942). *J. Immunol.* **45**, 159-170.
5. Weller, T.H. and Coons, A.H. (1954). *Proc. Soc. Exp. Biol.* (New York) **86**, 789-794.

# Selecting an ELISA Plate

**Choose a microplate with a minimum protein-binding capacity of 400ng/cm². It is also important that the CV value (coefficient of variation) of the protein binding be below 5%.** Thermo Scientific ELISA Plates undergo extensive quality testing and controlled manufacture and are guaranteed to have CVs less than 5%. Use Thermo Scientific Pierce 8-Well Strip Plates (Product # 15031) when partial-plate assays are performed frequently. The choice of plate color depends upon the signal being detected. Clear polystyrene plates are used for colorimetric signals and black or white opaque plates are used for fluorescent and chemiluminescent signals.

### Characteristics:

- Antibody Binding: High, 400ng IgG/cm² (1cm² = 100µL vol.)
- Binding Interaction: Hydrophobic/Ionic (-)
- Performance Certified: Yes. Well-to-well, CV ≤ 3%
- Well Shape (bottom): Flat
- Maximum Well Volume: 360µL
- Packaging: 1 plate per sealed tray
- Units per package: 100 plates

## Reagent Reservoirs

*Allows easy dispensing of reagents by multi-channel pipettors.*

### Highlights:

- Sterile, sturdy reservoirs are molded from high-impact polystyrene
- Thermo Scientific Reservoirs facilitate repeated pick up of reagents by multi-channel pipettors for delivery to 96-well plates

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 15075 | Reagent Reservoirs<br>Sterile, 50mL capacity | 200/pkg |

## Sealing Tape for 96-Well Plates

### Highlights:

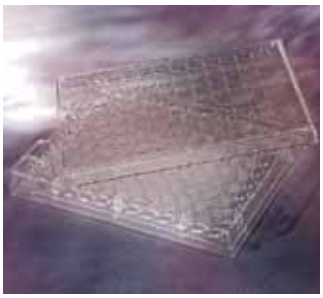
- Seal plates quickly and easily to allow mixing or prevent evaporation

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 15036 | Sealing Tape for 96-Well Plates<br>Pre-cut pressure-sensitive sealing tape | 100/pkg |

## 96-Well Plates

*Enhance antibody-binding properties in your ELISA applications.*



### Highlights:

- Thermo Scientific Pierce Plates are made of specially formulated polystyrene and treated to produce high antibody-binding characteristics
- Each lot manufactured is certified to ensure adherence to strict performance criteria

### Characteristics:

- Antibody Binding: High, 400ng IgG/cm$^2$ (1cm$^2$ = 100µL vol.)
- Binding Interaction: Hydrophobic/Ionic
- Performance Certification: Well-to-well, CV ≈ 3%
- Well Shape (bottom): Flat
- Maximum Well Volume: 360µL
- Packaging: 1 plate per sealed tray

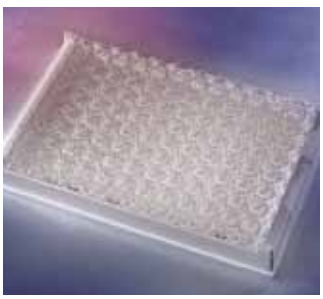
**Reference**
Foster, B.A., *et al.* (1999). *Science* **286**, 2507-2510.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **15041** | 96-Well Plates – Corner Notch | 100/pkg |
| **15042** | White Opaque 96-Well Plates | 25/pkg |

## 8-Well Strip Plates

*No more loose-fitting strips!*



### Highlights:

- Special optically clear polystyrene formulation
- High antibody-binding surface improves the overall performance in immunoassay applications
- Thermo Scientific Pierce Plates are certified for performance
- Consistent low coefficients of variation
- Eight-well strips fit snugly in the plate frame, virtually eliminating strip loss while in use – even if turned upside down
- Strip guides help to avoid misorientation of the strip when returned to the holder
- Strips are gently removed from the frame with a strip well ejector
- Wells can be easily separated from the strips with a gentle twist, allowing you to work with individual wells or strips of less than eight wells

### Characteristics:

- Antibody Binding: High, 400ng IgG/cm$^2$ (1cm$^2$ = 100µL vol.)
- Binding Interaction: Hydrophobic/Ionic
- Performance Certification: Well-to-well, CV ≈ 3%
- Well Shape (bottom): Flat
- Maximum Well Volume: 360µL
- Packaging: 5 plates per sealed tray

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **15031** | Pierce® 8-Well Plates – Corner Notch<br>Includes one Strip Well Ejector per package. | 100/pkg |

## DNA Coating Solution

*Quickly deposit DNA onto a plastic surface for hybridization.*

Thermo Scientific Pierce DNA Coating Solution allows efficient DNA surface coating of microplates and micro-sample tubes.

### Highlights:

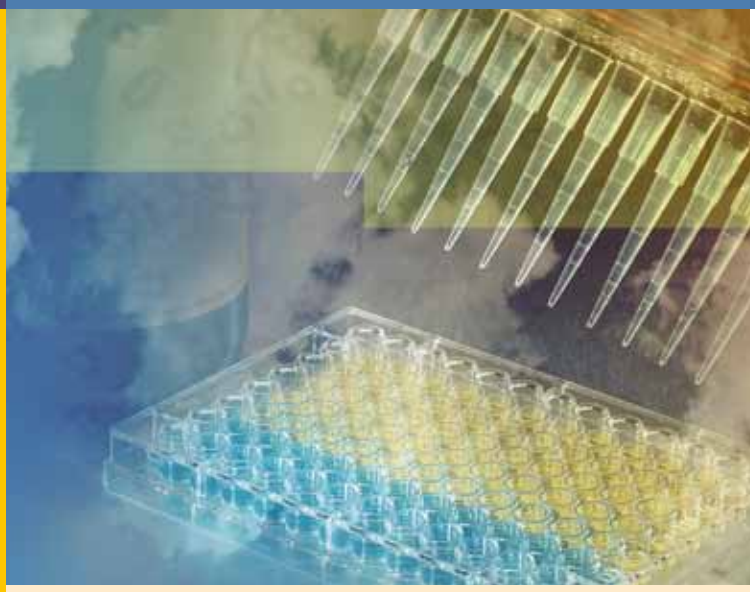
- No more overnight incubations or heating microplates and waiting
- Promotes high-efficiency coating of DNA onto polystyrene or polypropylene surfaces – hybridization efficiency is related to efficiency of DNA surface immobilization.[1]
- Target DNA coated onto microplates permits speed and flexibility in probing
- DNA coated onto plastic surfaces is stabilized sufficiently to allow storage for months
- DNA Coating Solution is autoclaved to inactivate DNase

**References**
1. Hirayama, H., *et al.* (1996). *Nucleic Acids Res.* **24,** 4098-4099.
2. van Gijssel, H.E., *et al.* (2002). *Cancer Epidem. Biomarker Prev.* **11**, 1622-1629.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **17250** | DNA Coating Solution<br>Sufficient for coating 5 x 96-well microplates (at 200µL/well). | 100mL |

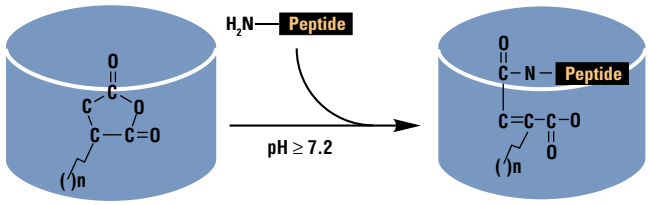
# Selecting an ELISA Plate



**There are several important points to consider when coating an ELISA plate. It is important to ensure that the coating solution is free of detergents because detergents often compete for binding and cause low and/or uneven binding.** For competitive assays, a lower coating concentration usually is chosen to ensure that the antibody is the limiting factor. Oddly, excessive concentrations of coating protein occasionally lead to less binding, a phenomenon known as the "hook" effect. A typical protein-coating solution concentration of 2-10µg/mL is a good starting range for successful plate-coating.

Smaller molecules such as peptides and many drugs require chemically activated microplates such as Thermo Scientific Pierce Maleic Anhydride Activated Plates or Sulfhydryl-binding Maleimide Activated Plates for attachment. The maleic anhydride group reacts with primary amines (lysine, N-terminus) and the maleimide reacts with sulfhydryl groups (–SH, reduced cysteine residues), forming a covalent bond (Figure 4). To increase the dynamic range of an assay for small molecules, they may be biotinylated and attached to a Thermo Scientific NeutrAvidin HBC (High Binding Capacity) Plate or a Thermo Scientific Pierce Streptavidin HBC Plate (Figure 5). These plates allow high binding biotinylated oligos, peptides, etc. to the surface of the well, and are provided as clear, black or white opaque plates and in 96- or 384-well plate formats.

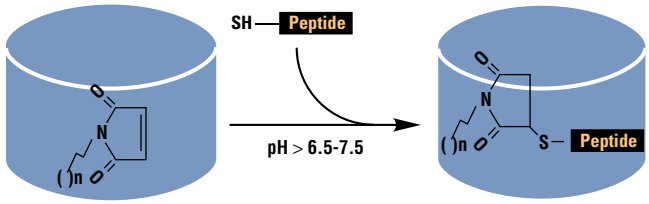
**Reaction Scheme for Coupling both Large and Small Amine-Containing Molecules**




Maleic Anhydride shown anchored to polystyrene well of a microplate. Both the well wall and base are activated.

Stable amide bond formation with amines, with a resulting hydrophilic well surface.

**Reaction Scheme for Coupling both Large and Small Sulfhydryl-Containing Molecules**




Maleimide shown anchored to polystyrene well of a microplate. Both the well wall and base are activated.

Stable thioether bond formation with sulfhydryls, with a resulting hydrophilic well surface.

**Figure 4. Coupling reactions of Thermo Scientific Pierce Activated Plates.**

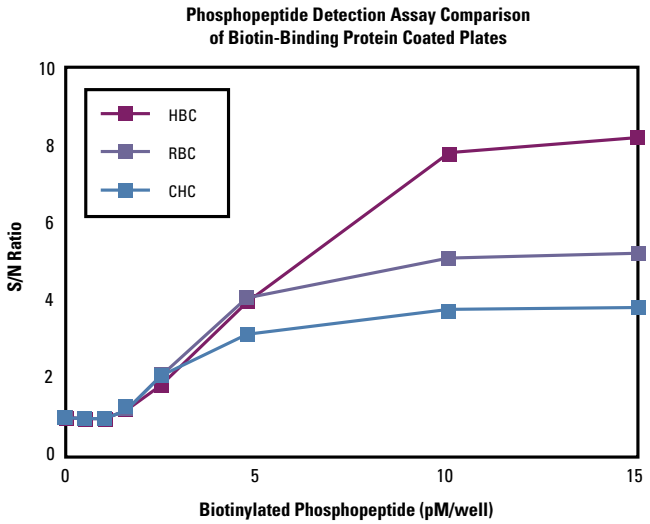



**Figure 5. Comparison of Thermo Scientific NeutrAvidin High Binding Capacity (HBC) Coated Plate, NeutrAvidin Regular Binding Capacity (RBC) Coated Plates and a competitor's Streptavidin Coated High Binding Capacity Plates (CHC).** Plates were incubated with various dilutions of biotinylated, phosphorylated peptide. After washing, the plates were incubated with mouse anti-phosphotyrosine antibody (1:1,000) and then detected using an anti-mouse-FITC conjugate (1:667). S/N = signal-to-noise ratio.

The initial step in many microplate-based assays involves passive adsorption of proteins to the plate wells. Several problems arise from passive adsorption, including improper orientation, denaturation, poor immobilization efficiency and binding of contaminants along with the target molecule. Our Coated Plates are designed to circumvent these problems. Antibodies can be attached to a microplate through the Fc region using Protein A, G, or A/G coated plates, which orients them properly and preserves their antigen-binding capability. Fusion proteins can be attached to a microplate in the proper orientation using our Glutathione or Metal Chelate. Because the polystyrene surface of our Coated Plates is precoated, immobilized proteins are physically separated from the surface and protected from its denaturing effects. Peptides and other small molecules, which are typically difficult to immobilize, can be biotinylated and attached to a Streptavidin or NeutrAvidin Coated Plate with high efficiency. Precoated plates bind selectively to the desired target proteins, minimizing any contamination from other molecules that are present in the preparation.

Studies report that the binding of antibodies to a microplate surface causes some denaturation.[1] This can be solved in several ways. Antibodies can be bound to a binding protein, such as Protein A, Protein G, Protein A/G or Protein L, that is coated on the plate. Alternatively, the antibody may be biotinylated and immobilized onto Streptavidin or NeutrAvidin Coated Plates. Either method physically separates the antibody from the surface of the plate for better assay performance.

Histidine- and glutathione S-transferase (GST)-tagged fusion proteins are popular for manipulating recombinantly expressed proteins. Developing ELISAs to detect the expression levels of these tagged proteins is simplified with microplates that are precoated with the ligand for capturing the fusion protein to be measured. Our Precoated Plates save time in developing ELISAs by 1) capturing the fusion protein from a crude cell lysate, 2) separating the fusion protein from the surface of the microplate to prevent denaturation and 3) properly orienting the fusion protein for detection. Table 1 lists the variety of Pierce Coated Plates available and the ligands they bind.

**Table 1. Thermo Scientific Pierce Coated Polystyrene Microplates.**

| | |
|---|---|
| **Protein A, G or A/G** | For binding antibodies via their Fc regions |
| **Protein L** | For binding Fab antibody fragments and single-chain variable fragments (ScFvs) through the kappa light chain |
| **Secondary Antibodies** | For binding antibodies, as an alternative to Protein A, G or L |
| **NeutrAvidin Protein or Streptavidin** | For binding biotinylated proteins, peptides or nucleic acids; also available in black or white opaque microplates |
| **Biotin** | For binding avidin, streptavidin or NeutrAvidin Biotin-Binding Protein |
| **$Ni^{2+}$ or Glutathione** | For binding recombinantly expressed proteins containing polyhistidine or glutathione S-transferase |
| **Maleic Anhydride** | For binding large or small amine-containing molecules |
| **Maleimide Activated** | For binding sulfhydryl-containing molecules |
| **Anti-GST** | For capturing proteins expressing glutathione S-transferase (GST) |

We also offer a custom plate-coating service for large batches of coated plates. Our automated plate-coating technology produces coated plates that will meet your quality specifications. Take advantage of our coating expertise to expedite the development of your plate-based assay. See pages 69-70 for more information on custom-coated plates.

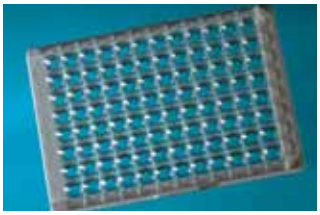
**Reference**

1. Schwab, C. and Bosshard, H.R. (1992). *J. Immunol. Method* **147**, 125-134.

# Selecting an ELISA Plate

## Protein A, G and A/G Coated Plates

*Bind antibodies through their Fc portion, correctly orienting them for maximum antigen capture.*



### Highlights:

- Retain antibody activity, which can be lost when antibodies are immobilized by passive adsorption
- Orient antibodies for maximum antigen-binding capacity
- Immobilize antibodies without prior purification
- Ensure minimal variation (< 5% well-to-well) from consistent coating
- Reduce nonspecific binding because plates are pre-blocked with SuperBlock Blocking Buffer
- Perform multiple immunoprecipitations
- Binds up to 2.5µg antibody/well (100µL coating volume)

### Protein A/G

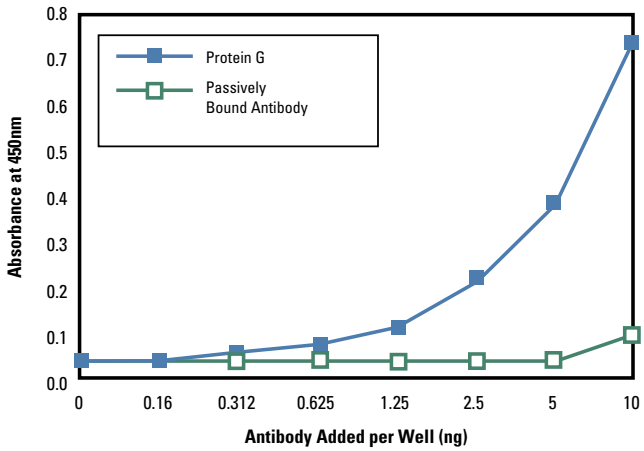
- Consists of a fusion protein containing four antibody-binding sites from Protein A and two from Protein G
- Binds to the broadest spectrum of antibodies because it combines the specificities of Protein A and Protein G
- Binds to Fc region of antibodies, ensuring optimal orientation
- Binds well using a wide range of pH

### Protein A

- Binds strongly to IgG from rabbit, guinea pig, pig, dog and rhesus monkey
- Binds strongly to mouse $IgG_{2a}$, $IgG_{2b}$ and $IgG_3$
- Binds to Fc region of antibodies for optimal orientation

### Protein G

- Binds strongly to IgG from many species including human, mouse, rabbit, sheep and goat
- Binds only to IgG – no cross-reactivity with other antibody classes
- Binds to Fc region of antibodies for optimal orientation




**Properly oriented antibodies retain higher activity.**


**References**
Desai, S. and Hermanson, G. (1997). *Previews* **1(3)**, 2-7.
**Protein A Coated Plate References**
Asthagiri, A.R., *et al.* (1999). *J. Biol. Chem.* **274**, 27119-27127.
Lawrenson, I.D., *et al.* (2002). *J. Cell Sci.* **115**, 1059-1072.
**Protein G Coated Plate References**
Lai, Z., *et al.* (2002). *Proc. Natl. Acad. Sci.* **99**, 14734-14739.
Rauch, J., *et al.* (2003). *J. Biol. Chem.* **278**, 47508-47515.


## Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15130** | Protein A, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 4pmol rabbit IgG/well | 5 plates |
| **15132** | Protein A, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 4pmol rabbit IgG/well | 5 plates |
| **15154** | Protein A, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 4pmol rabbit IgG/well | 5 plates |
| **15155** | Protein A, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 4pmol rabbit IgG/well | 5 plates |
| **15131** | Protein G, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 2pmol rabbit IgG/well | 5 plates |
| **15133** | Protein G, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 2pmol rabbit IgG/well | 5 plates |
| **15156** | Protein G, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 2pmol rabbit IgG/well | 5 plates |
| **15157** | Protein G, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 2pmol rabbit IgG/well | 5 plates |
| **15138** | Protein A/G, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 5pmol rabbit IgG/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

## Protein L Coated Plates

*Great for binding scFv and Fab fragments.*

Protein L is an immunoglobulin-binding protein that has the unique ability to bind through kappa light chain interactions without interfering with an antibody's antigen-binding site. This gives Protein L the ability to bind a wider range of Ig classes and subclasses than other antibody-binding proteins such as Protein A or Protein G. This also gives Protein L the unique ability to bind single-chain variable fragments (scFv) and Fab fragments.

### Highlights:

- Binds to all classes of Ig (IgG, IgM, IgA, IgE and IgD)
- Binds to the $V_L$ region of kappa light chains (human I, III and IV and mouse I) without interfering with antigen-binding sites
- Binds scFv
- Does not bind bovine, goat or sheep Ig
- Binds weakly to rabbit Ig
- Pre-blocked with SuperBlock Buffer to reduce nonspecific binding


**References**

Åkerström, B. and Björck, L. (1989). *J. Biol. Chem.* **264**, 19740-19746.
Björck, L. (1988). *J. Immunol.* **140**, 1194-1197.
Kastern, W., *et al.* (1992). *J. Biol. Chem.* **267**, 12820-12825.
Nilson, B.H.K., *et al.* (1993). *J. Immunol. Method* **164**, 33-40.
Rhee, J.,*et al.* (2001). *J. Biol. Chem.* **276**, 6640-6644.


### Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15190** | Protein L, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 3pmol mouse IgG/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

## Antibody Coated Plates

*For species-specific antibody capture.*

### Highlights:

- Prevents antibody denaturation as a result of direct binding to polystyrene
- Unlike Protein A or Protein G Plates, Thermo Scientific Pierce Antibody Coated Plates bind only to target species
- Antibody-binding capacity is higher than direct adsorption onto polystyrene
- Pre-blocked with SuperBlock Buffer to minimize nonspecific binding


**References**

Gartner, W., *et al.* (2001). *Cereb Cortex.* **11**, 1161-1169.
Wagner, L., *et al.* (2000). *J. Biol. Chem.* **275**, 24740-24751.


### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **Goat Anti-Mouse Coated Plates** | | |
| **15134** | Clear 96-Well Plates | 5 plates |
| **15234** | White 96-Well Plates | 5 plates |
| **15334** | Black 96-Well Plates | 5 plates |
| **Goat Anti-Rabbit Coated Plates** | | |
| **15135** | Clear 96-Well Plates | 5 plates |
| **15136** | White 96-Well Plates | 5 plates |
| **15137** | Black 96-Well Plates | 5 plates |

## NeutrAvidin Coated Plates

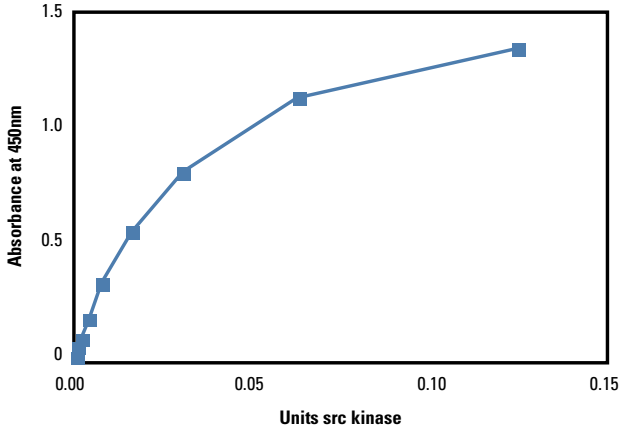
*The same high affinity of avidin for biotin, without the nonspecific binding problems.*

### Highlights:

- Easy and gentle immobilization of biotin-containing conjugates
- Lowest nonspecific binding properties of all biotin-binding proteins
- NeutrAvidin Biotin-Binding Protein has no carbohydrate and an isoelectric point of 6.3
- No denaturing of the protein component of a conjugate upon binding
- Ideal for binding small hydrophilic molecules (e.g., peptides) that typically exhibit poor binding directly to polystyrene
- Pre-blocked with your choice of Blocker BSA or SuperBlock Blocking Buffer
- Available in 96-well and 384-well formats

**Characteristics of biotin-binding proteins.**

| Protein | Isoelectric Point | Contains Carbohydrate | Nonspecific Binding |
|---|---|---|---|
| Avidin | 10-10.5 | Yes | High |
| Streptavidin | 5 | No | Low |
| NeutrAvidin Protein | 6.3 | No | Ultralow |




**Purified p60$^{c\text{-}src}$ activity detection with TK peptide 2.** Biotinylated tyrosine kinase peptide 2 was added to Thermo Scientific NeutrAvidin Coated Plates and incubated for 30 minutes. Wells were washed; samples containing p60$^{c\text{-}src}$ tyrosine kinase were added to phosphorylate the tyrosine residue on the peptide. Anti-phosphotyrosine monoclonal antibody conjugated to HRP was added. Tyrosine kinase activity was detected by Thermo Scientific 1-Step Turbo TMB Substrate. Kinase activity was quantitated by comparison with a standard curve generated using the phosphorylated form of the same peptide substrate.

**Thermo Scientific NeutrAvidin Coated Plate characteristics.**

| | 96-Well Plate | 384-Well Plate |
|---|---|---|
| Binding Capacity | 15pmol/well | 10pmol/well |
| Coat Volume | 100µL/well | 50µL/well |
| Blocking Volume | 200µL/well | 100µL/well |

**References**
Brett, P.J., *et al.* (2002). **277**, 20468-20476.
Denlinger, L.C., *et al.* (2001). *J. Immunol.* **167**, 1871-1876.
Guixiang Dai, G., *et al.* (2002). *J. Biol. Chem.* **277**, 161-168.
Patil, S., *et al.* (1999). *J. Biol. Chem.* **274**, 28575-28583.
Singh, Y., *et al.* (1999). *Infect. Immun.* **67**, 1853-1859.

## Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15129** | NeutrAvidin Protein, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 15pmol biotin/well | 5 plates |
| **15127** | NeutrAvidin Protein, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 15pmol biotin/well | 5 plates |
| **15400** | NeutrAvidin Protein, 100µL | Clear, 384-Well | SuperBlock BB, 100µL | ~ 10pmol biotin/well | 5 plates |
| **15116** | NeutrAvidin Protein, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 15pmol biotin/well | 5 plates |
| **15401** | NeutrAvidin Protein, 50µL | White, 384-Well | SuperBlock BB, 100µL | ~ 10pmol biotin/well | 5 plates |
| **15117** | NeutrAvidin Protein, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 15pmol biotin/well | 5 plates |
| **15402** | NeutrAvidin Protein, 50µL | Black, 384-Well | SuperBlock BB, 100µL | ~ 10pmol biotin/well | 5 plates |
| **15123** | NeutrAvidin Protein, 200µL | Clear, 96-Well | Blocker BSA, 300µL | ~ 15pmol biotin/well | 5 plates |
| **15128** | NeutrAvidin Protein, 200µL | Clear, 8-Well Strip | Blocker BSA, 300µL | ~ 15pmol biotin/well | 5 plates |
| **15216** | NeutrAvidin Protein, 200µL | White, 96-Well | Blocker BSA, 300µL | ~ 15pmol biotin/well | 5 plates |
| **15217** | NeutrAvidin Protein, 200µL | Black, 96-Well | Blocker BSA, 300µL | ~ 15pmol biotin/well | 5 plates |
| **15115** | Pierce Biotin Binding Plate Sample Pack, one each of # 15120, 15121, 15127, 15128 | | | | 4 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

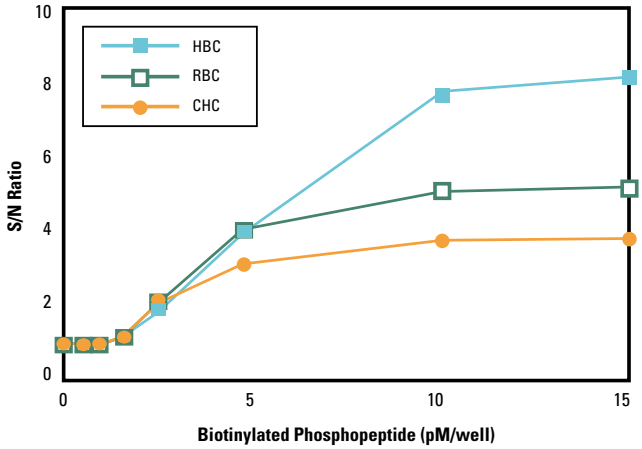
## NeutrAvidin High Binding Capacity (HBC) Coated Plates

*Unique technology for improved assay precision.*

Our plate-coating technology produces a Thermo Scientific NeutrAvidin HBC Plate with a wider detection limit than our regular binding capacity plates. The standard curve exhibits greater linearity for detecting small biotinylated molecules such as peptides and oligonucleotides, resulting in greater assay precision. Switch your assay over to our NeutrAvidin HBC Coated Plates for binding small biotinylated ligands and see the difference for yourself.

### Highlights:

- Unique plate-coating technology – results in high loading of NeutrAvidin Biotin-Binding Protein/well
- Improved sensitivity – less nonspecific binding for improved signal:noise ratios
- Broader dynamic range – extends the quantitative range so there is no need for dilutions
- Save time – pre-blocked plate reduces the number of assay steps
- Flexible assay formats – coated plates offered in 96- and 384-well formats and in different colors




**Phosphopeptide detection assay comparison of biotin-binding protein-coated plates.** Comparison of Thermo Scientific NeutrAvidin High Binding Capacity (HBC) Coated Plate, NeutrAvidin Regular Binding Capacity (RBC) Coated Plates and a competitor's Streptavidin Coated High Binding Capacity Plates (CHC). Plates were incubated with various dilutions of biotinylated, phosphorylated peptide. After washing, the plates were incubated with mouse anti-phosphotyrosine antibody (1:1000) and then detected using an anti-mouse-FITC conjugate (1:666). S/N = single to noise.

**Thermo Scientific NeutrAvidin HBC Coated Plate characteristics.**

| | 96-Well Plate | 384-Well Plate |
|---|---|---|
| Binding Capacity | 60pmol/well | 35pmol/well |
| Coat Volume | 100µL/well | 50µL/well |
| Blocking Volume | 200µL/well | 100µL/well |

## Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15507** | NeutrAvidin Protein, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 60pmol biotin/well | 5 plates |
| **15508** | NeutrAvidin Protein, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 60pmol biotin/well | 5 plates |
| **15511** | NeutrAvidin Protein, 50µL | Clear, 384-Well | SuperBlock BB, 100µL | ~ 35pmol biotin/well | 5 plates |
| **15509** | NeutrAvidin Protein, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 60pmol biotin/well | 5 plates |
| **15512** | NeutrAvidin Protein, 50µL | White, 384-Well | SuperBlock BB, 100µL | ~ 35pmol biotin/well | 5 plates |
| **15510** | NeutrAvidin Protein, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 60pmol biotin/well | 5 plates |
| **15513** | NeutrAvidin Protein, 50µL | Black, 384-Well | SuperBlock BB, 100µL | ~ 35pmol biotin/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

# Selecting an ELISA Plate

## Streptavidin Coated Plates

*The specific binding affinity of streptavidin for biotin – in a microplate.*

### Highlights:

- Easy and gentle immobilization of biotin-containing conjugates
- Low nonspecific binding
- No denaturing of the biotinylated protein upon binding
- Ideal for binding small biotinylated hydrophilic molecules (e.g., peptides) that typically exhibit poor binding to polystyrene
- Pre-blocked with your choice of Blocker BSA or SuperBlock Blocking Buffer

**Thermo Scientific Pierce Streptavidin Coated Plate characteristics.**

| | 96-Well Plate | 384-Well Plate |
|---|---|---|
| Binding Capacity | 5pmol/well | 4pmol/well |
| Coat Volume | 100µL/well* | 50µL/well |
| Blocking Volume | 200µL/well | 100µL/well |

** Product # 15125 is coated with 200µL of streptavidin coating solution.*


**References**

Estrada, G., *et al.* (1996). *Mol. Cell Probes* **10**, 179-185.
Grobler, J.A., *et al.* (2002). *Proc. Natl. Acad. Sci.* **99**, 6661-6666.
Hong, P. W.-P., *et al.* (2002). *J. Virol.* **76**, 12855-12865.
Stühlinger, M.C.,*et al.* (2001). *Circulation* **104**, 2569-2575.
Su, S.V., *et al.* (2004). *J. Biol. Chem.* **279**, 19122-19132.


### Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15124** | Streptavidin, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 5pmol biotin/well | 5 plates |
| **15126** | Streptavidin, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 5pmol biotin/well | 5 x 5 plates |
| **15120** | Streptavidin, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 5pmol biotin/well | 5 plates |
| **15122** | Streptavidin, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 5pmol biotin/well | 5 x 5 plates |
| **15405** | Streptavidin, 50µL | Clear, 384-Well | SuperBlock BB, 100µL | ~ 4pmol biotin/well | 5 plates |
| **15118** | Streptavidin, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 5pmol biotin/well | 5 plates |
| **15119** | Streptavidin, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 5pmol biotin/well | 5 plates |
| **15407** | Streptavidin, 50µL | Black, 384-Well | SuperBlock BB, 100µL | ~ 4pmol biotin/well | 5 plates |
| **15125** | Streptavidin, 200µL | Clear, 96-Well | Blocker BSA, 300µL | ~ 10pmol biotin/well | 5 plates |
| **15121** | Streptavidin, 200µL | Clear, 8-Well Strip | Blocker BSA, 300µL | ~ 10pmol biotin/well | 5 plates |
| **15218** | Streptavidin, 200µL | White, 96-Well | Blocker BSA, 300µL | ~ 10pmol biotin/well | 5 plates |
| **15219** | Streptavidin, 200µL | Black, 96-Well | Blocker BSA, 300µL | ~ 10pmol biotin/well | 5 plates |
| **15115** | Biotin Binding Plate Sample Pack, one each of # 15120, 15121, 15127, 15128 | | | | 4 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

## Biotin Coated Plates

*A simple format for testing the efficiency of avidin, streptavidin or NeutrAvidin Protein conjugations.*

Thermo Scientific Pierce Biotin Coated Plates can be used in any immunoassay with NeutrAvidin Biotin-Binding Protein, streptavidin, avidin or other biotin-binding proteins.

### Highlights:

- Biotin group accessible for binding avidin, streptavidin or NeutrAvidin Biotin-Binding Protein
- Pre-blocked to reduce nonspecific binding
- Strip well plate format for the ultimate in convenience
- Coat volume: 200µL
- Blocking volume: 300µL

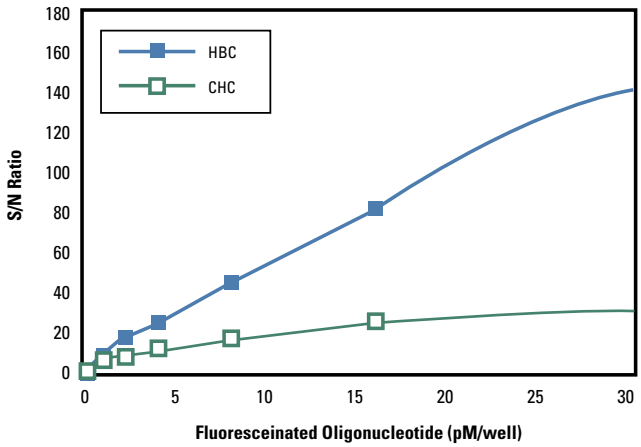
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **15151** | Pierce Biotin Coated Clear 8-Well Strip Plates | 5 plates |

## Streptavidin HBC Coated Plates

*Take advantage of our technology that provides a broader dynamic range.*

Thermo Scientific Pierce Streptavidin High Binding Capacity (HBC) Coated Plates are designed for binding biotinylated oligonucleotides and peptides with higher binding efficiency than other commercially available plates. Our proprietary coating technology has created a streptavidin-coated plate with four- to five-times the binding capacity of competitors' plates. Using our Streptavidin HBC Plate can result in an assay with a broader dynamic range and better linearity, for improved assay precision. The figure illustrates this effect when measuring fluorescent polymerase chain reaction (PCR) products hybridized to biotinylated oligonucleotides bound to our Streptavidin High Binding Capacity (HBC) Plate and a competitor's high binding capacity plate (CHC). Try the Pierce Streptavidin HBC Coated Plate and see what has been going undetected in your research.




**Comparison of Thermo Scientific Pierce Streptavidin High Binding Capacity (HBC) and competitor's HBC coated plates.** Fluoresceinated oligonucleotide hybridization assay comparison. Comparison of Pierce Streptavidin High Binding Capacity (HBC) Coated Plate with competing high binding capacity plate (CHC). Plates were incubated with a biotinylated oligonucleotide, washed and probed with a complementary oligonucleotide labeled with fluorescein at various dilutions. S/N = signal:noise ratio.

### Highlights:

- Broader dynamic range – extends the quantitative range so there's no need for dilutions
- Better sensitivity – increased binding capacity allows direct detection of small ligands not observed with regular binding capacity plates
- Superior assay precision – standard curve demonstrates greater linearity
- Save time – pre-blocked to reduce number of assay steps
- Flexible assay formats – offered in 96- and 384-well formats and in different colors

**Thermo Scientific Pierce Streptavidin HBC Coated Plate characteristics.**

| | 96-Well Plate | 384-Well Plate |
|---|---|---|
| Binding Capacity | 125pmoles/well | 60pmol/well |
| Coat Volume | 100µL/well | 50µL/well |
| Blocking Volume | 200µL/well | 100µL/well |

**Reference**
Wilson, D.S., *et al.* (2001). *Proc. Natl. Acad. Sci.* **98**, 3750-3755.

## Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15500** | Streptavidin, 100µL | Clear, 96-Well | SuperBlock BB, 200µL | ~ 125pmol biotin/well | 5 plates |
| **15501** | Streptavidin, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 125pmol biotin/well | 5 plates |
| **15504** | Streptavidin, 50µL | Clear, 384-Well | SuperBlock BB, 100µL | ~ 60pmol biotin/well | 5 plates |
| **15502** | Streptavidin, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 125pmol biotin/well | 5 plates |
| **15505** | Streptavidin, 50µL | White, 384-Well | SuperBlock BB, 100µL | ~ 60pmol biotin/well | 5 plates |
| **15503** | Streptavidin, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 125pmol biotin/well | 5 plates |
| **15506** | Streptavidin, 50µL | Black, 384-Well | SuperBlock BB, 100µL | ~ 60pmol biotin/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

# Selecting an ELISA Plate

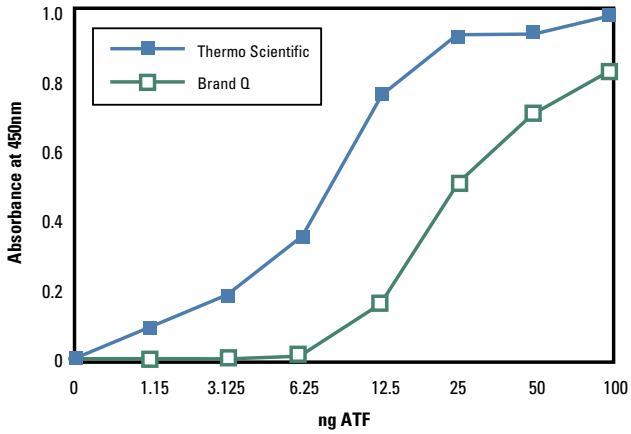
## Nickel Coated Plates

*Bind recombinantly expressed fusion proteins containing a histidine tag for easy ELISA analysis.*

Thermo Scientific Pierce $Ni^{2+}$ Chelate-coated Plates provide a simple format for protein:protein interaction studies.

### Highlights:

- Detergents used to lyse cells do not inhibit binding to $Ni^{2+}$- coated plates as they do with plain polystyrene
- The detection limit is 1ng of histidine fusion protein
- Better binding for more sensitive assays compared to other commercially available nickel-activated plates[1]
- Can be custom-made with other metals. Contact Bulk & Custom Sales or your local distributor




**Binding comparison of histidine-tagged ATF fusion protein.**


**References**

1. Desai, S., *et al.* (1997). *Previews* **1(4)**, 12-15.
   Pan, W., *et al.* (2003). *J. Biol. Chem.* **278**, 27820-27827.
   Tu, Y., *et al.* (1999). *Mol. Cell. Biol.* **19**, 2425-2434.


## Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15442** | Nickel Chelate, 200µL | Clear, 96-Well | BSA, 200µL | ~ 9pmol His-tagged protein/well | 5 plates |
| **15142** | Nickel Chelate, 200µL | Clear, 8-Well Strip | BSA, 200µL | ~ 9pmol His-tagged protein/well | 5 plates |
| **15242** | Nickel Chelate, 200µL | White, 96-Well | BSA, 200µL | ~ 9pmol His-tagged protein/well | 5 plates |
| **15342** | Nickel Chelate, 200µL | Black, 96-Well | BSA, 200µL | ~ 9pmol His-tagged protein/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*

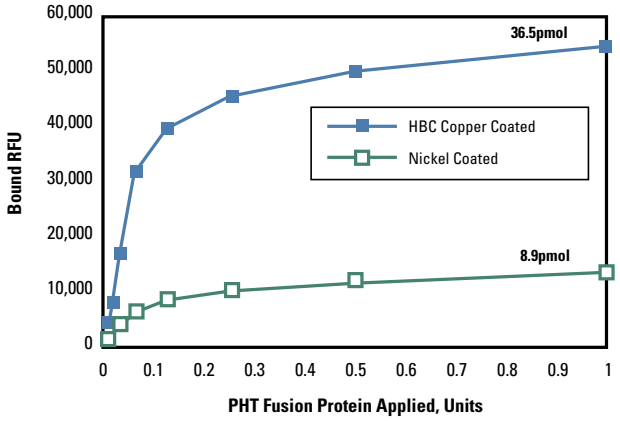
## Copper Coated High Binding Capacity (HBC) Plates

*Boost assay performance by binding more histidine-tagged protein.*

Thermo Scientific Pierce Copper Coated High Binding Capacity (HBC) Plates use an exclusive coating process to increase the amount of histidine-tagged protein that will bind to the plate surface. This is ideal for high-throughput screening applications that need improved sensitivity and greater dynamic range.

### Highlights:

- Quantitate previously undetectable histidine-tagged proteins
- Wider dynamic range with four-fold greater capacity than regular nickel chelate-coated plates
- Copper chelate provides greater binding capacity




**Binding comparison of a histidine-tagged fluorescent fusion protein to standard Thermo Scientific Pierce Nickel Coated and Copper Coated High Binding Capacity (HBC) Plates.** Pierce Copper Coated HBC Plates exhibit a four-fold greater capacity for binding purified polyhistidine-tagged protein when assayed using a 100µL volume. Incubation time was two hours for binding.

## Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15143** | Copper Chelate, 200µL | Clear, 96-Well | BSA, 200µL | ~ 35pmol His-tagged protein/well | 5 plates |
| **15146** | Copper Chelate, 200µL | Clear, 8-Well Strip | BSA, 200µL | ~ 35pmol His-tagged protein/well | 5 plates |
| **15147** | Copper Chelate, 200µL | White, 96-Well | BSA, 200µL | ~ 35pmol His-tagged protein/well | 5 plates |
| **15148** | Copper Chelate, 200µL | Black, 96-Well | BSA, 200µL | ~ 35pmol His-tagged protein/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*

# Selecting an ELISA Plate

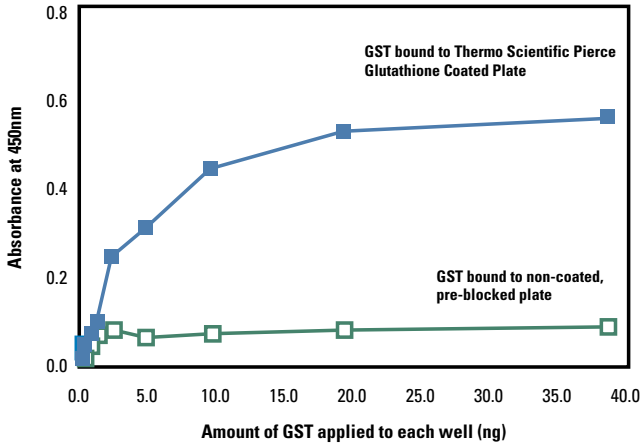
## Glutathione Coated Plates

*Bind fusion proteins containing GST.*

Thermo Scientific Pierce Glutathione Coated Plates provide easy quantitation of antibodies against GST-fused proteins.

### Highlights:

- Simple format for protein:protein interaction studies
- Detergents used to lyse cells don't inhibit binding to precoated plates as they do with plain polystyrene
- Pre-blocked with SuperBlock Buffer to reduce nonspecific binding





**References**

McKevitt, M., *et al.* (2003). *Genome Res.* **13**, 1665-1674.
Pullen, S.S., *et al.* (1999). *J. Biol. Chem.* **274**, 14246-14254.
Yarwood, S.J., *et al.* (1999). *J. Biol. Chem.* **274**, 14909-14917.


### Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15140** | Glutathione, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 10ng GST protein/well | 5 plates |
| **15240** | Glutathione, 100µL | White, 96-Well | SuperBlock BB, 200µL | ~ 10ng GST protein/well | 5 plates |
| **15340** | Glutathione, 100µL | Black, 96-Well | SuperBlock BB, 200µL | ~ 10ng GST protein/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

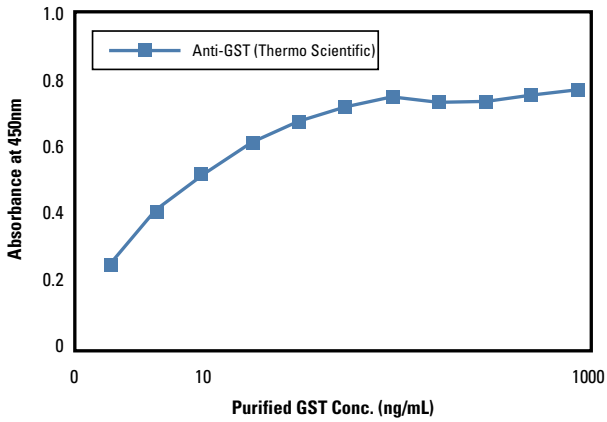
## Anti-GST Coated Plates

*A unique alternative to glutathione-coated plates for binding GST fusion proteins.*

Anti-GST antibodies often provide better binding of GST fusion proteins because they bind improperly folded and denatured GST.

### Highlights:

- Binds native or denatured forms of GST; an alternative to glutathione-coated plates
- Pre-blocked with SuperBlock Buffer
- Thermo Scientific Pierce Cell Lysis Reagents will not interfere with GST fusion protein binding




**Thermo Scientific Pierce Anti-GST Coated Plates were tested for their ability to bind GST.** The plates were assayed with purified GST that was detected using a rabbit anti-GST antibody (1mg/mL) followed by donkey anti-rabbit HRP conjugate. The signal was developed with Thermo Scientific 1-Step Turbo TMB Substrate (Product # 34022).

### Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer** | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15144** | Goat Anti-GST Antibody, 100µL | Clear, 8-Well Strip | SuperBlock BB, 200µL | ~ 10ng GST protein/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*
*** BB=Blocking Buffer*

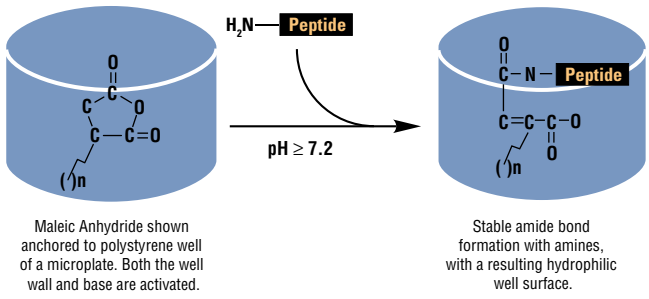
## Amine-binding Maleic Anhydride Plates

*Proteins and other primary amine-containing compounds covalently attach to the microplate.*

Great for immobilization of compounds that do not readily bind to plain polystyrene plates.

### Highlights:

- Spontaneously react with primary amines
- Maleic anhydride retains its integrity and coupling availability for months




Maleic Anhydride shown anchored to polystyrene well of a microplate. Both the well wall and base are activated.

Stable amide bond formation with amines, with a resulting hydrophilic well surface.

**Reaction scheme for coupling both large and small amine-containing molecules.**


**References**

Batista, F.D. and Neuberger, M.S. (2000). *EMBO J.* **19**, 513-520.
Brett, P.J., *et al.* (2002). *J. Biol. Chem.* **277,** 20468-20476.
Dumoutier, L., *et al.* (2001). *J. Immunol.* **166**, 7090-7095.
Leu, S.-J., *et al.* (2003). *J. Biol. Chem.* **278**, 33801-33808.
Liao, Y.-F., *et al.* (2002). *J. Biol. Chem.* **277**, 14467-14474.
Marston, E.L., *et al.* (2002). *Clin. Diagn. Lab. Immunol.* **9**, 446-452.


### Ordering Information

| Product # | Coating | Plate Type | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|
| **15110** | Maleic Anhydride, 200µL | Clear, 96-Well | ~ 125pmol biotin-pentylamine/well | 5 plates |
| **15112** | Maleic Anhydride, 200µL | Clear, 96-Well | ~ 125pmol biotin-pentylamine/well | 5 x 5 plates |
| **15100** | Maleic Anhydride, 200µL | Clear, 8-Well Strip | ~ 125pmol biotin-pentylamine/well | 5 plates |
| **15102** | Maleic Anhydride, 200µL | Clear, 8-Well Strip | ~ 125pmol biotin-pentylamine/well | 5 x 5 plates |
| **15108** | Maleic Anhydride, 200µL | White, 96-Well | ~ 125pmol biotin-pentylamine/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*

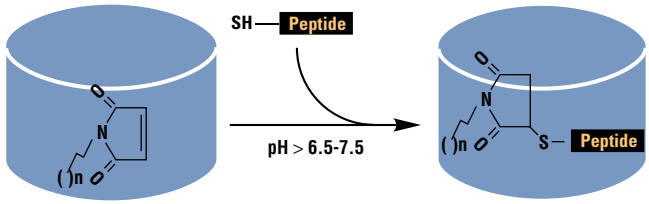
## Sulfhydryl-binding Maleimide Activated Plates

*A convenient alternative to amine-reactive chemistries for attaching sulfhydryl-containing compounds.*

Maleimide groups specifically and covalently conjugate sulfhydryl groups at neutral pH, creating a stable thioether bond.

### Highlights:

- Pre-blocked to reduce nonspecific binding
- Convenient 8-well strip format
- Easy (spontaneous) immobilization of peptides derivatized with a terminal cysteine and proteins with free sulfhydryl




Maleimide shown anchored to polystyrene well of a microplate. Both the well wall and base are activated.

Stable thioether bond formation with sulfhydryls, with a resulting hydrophilic well surface.

**Reaction scheme for coupling both large and small sulfhydryl-containing molecules.**


**References**

Ostrowski, M., *et al.* (2002). *J. Virol.* **76,** 4241-4250.
Reinhard, M., *et al.* (1999). *J. Biol. Chem.* **274**, 13410-13418.


### Ordering Information

| Product # | Coating | Plate Type | Blocking Buffer | Binding Capacity* | Pkg. Size |
|---|---|---|---|---|---|
| **15150** | Maleimide, 200µL | Clear, 8-Well Strip | BSA, 200µL | ~ 100-150pmol peptide-SH/well | 5 plates |
| **15152** | Maleimide, 200µL | White, 96-Well | BSA, 200µL | ~ 100-150pmol peptide-SH/well | 5 plates |
| **15153** | Maleimide, 200µL | Black, 96-Well | BSA, 200µL | ~ 100-150pmol peptide-SH/well | 5 plates |

** Approximate values; plates tested for specific signal:noise and C.V.*

# Blocking and Washing

## Blocking Unoccupied Sites

**In an ELISA, it is important to block the unoccupied sites on the surface of the well to reduce the amount of nonspecific binding of proteins during subsequent steps in the assay. A variety of blocking buffers ranging from nonfat milk to highly purified proteins have been used to block unreacted sites.** The blocking buffer should improve the sensitivity of the assay by reducing the background interference. An individual blocking buffer will not be compatible with every system; for this reason, a variety of blockers in both Tris-buffered saline (TBS) and phosphate-buffered saline (PBS) are available. The proper choice of blocker for a given assay depends on the antigen itself and on the type of enzyme conjugate to be used. For example, with applications using an alkaline phosphatase conjugate, a blocking buffer in TBS should be selected because PBS interferes with alkaline phosphatase. The ideal blocking buffer will bind to all potential sites of nonspecific interaction, eliminating background altogether, without altering or obscuring the epitope for antibody binding.

For true optimization of the blocking step for a particular immunoassay, empirical testing is essential. Many factors can influence nonspecific binding, including various protein:protein interactions unique to a given ELISA. The most important parameter when selecting a blocker is the signal:noise ratio, which is measured as the signal obtained with a sample containing the target analyte as compared to that obtained with a sample without the target analyte. Using inadequate amounts of blocker will result in excessive background and a reduced signal:noise ratio. Using excessive concentrations of blocker may mask antibody-antigen interactions or inhibit the enzyme, again causing a reduction of the signal:noise ratio. When developing any new ELISA, it is important to test several different blockers for the highest signal:noise ratio in the assay. No single blocking agent is ideal for every occasion because each antibody-antigen pair has unique characteristics.

We offer a complete line of Thermo Scientific Blocking Buffers for ELISA including StartingBlock, SuperBlock, Casein, BSA, SEA BLOCK and BLOTTO Blocking Buffers.

- Pierce Protein-Free Blocking Buffers contain a proprietary compound for blocking excess binding sites in ELISA, Western blotting, arrays and other immunodetection applications. This blocking buffer reduces or eliminates many of the problems encountered with traditional protein-blocking reagents, including cross-reactivity and interference from glycosylation. Additionally, Protein-Free Blocking Buffers are compatible with antibodies and avidin/biotin systems.
- StartingBlock Buffer contains no biotin or serum protein and is compatible with a wide range of detection systems. StartingBlock Buffer works almost immediately on ELISA plates, requiring no incubation step.
- SuperBlock Blocking Buffer is a highly purified non-serum protein solution that is the ideal blocking agent in many assays. The blocking ability of SuperBlock Buffers and their ability to maintain a high signal:noise ratio is superior to most other formulations. SuperBlock Blocking Buffers also accomplish blocking very quickly, often in as little as 10 minutes.
- Blocker Casein is a 1% (w/v) ready-to-use solution of Hammersten Grade casein that can be used to block nonspecific sites. Since casein is a purified protein, it is less likely than BLOTTO or other complex protein mixtures to cross-react with the antibodies and cause high background.
- Blocker BSA is a 10% solution of high-quality bovine serum albumin (BSA). BSA is a commonly used blocking agent for all immunoassay applications. Blocker BSA is concentrated and must be diluted before use. One to three percent BSA solutions are commonly used for blocking non-specific sites.
- SEA BLOCK Blocking Buffer is made from steelhead salmon serum. As a non-mammalian protein blocker, the risk of background caused by nonspecific interactions is minimized.
- Blocker BLOTTO is a ready-to-use blocking buffer made from nonfat dry milk. Nonfat dry milk contains endogenous biotin and should not be used with avidin-biotin systems.

## StartingBlock Blocking Buffer

*Thermo Scientific StartingBlock Blocking Buffer simplifies the selection of a blocker for ELISA applications.*

Although no blocking buffer is ideal for every system, you can improve the odds dramatically with our StartingBlock Blocking Buffer because it is compatible with the widest variety of antibodies.

For example: StartingBlock Blocking Buffers are compatible with biotin-containing systems, while milk-based protein blockers interfere. Our StartingBlock Buffers do not cross-react with rabbit antibodies, while many other blockers do. StartingBlock Blocking Buffers are also free of potentially interfering serum proteins.

Our StartingBlock Blocking Buffers offer a high level of performance – regardless of the system you choose for your ELISA or Western blotting application. In fact, they may be the only blockers you ever use.

### Highlights:

**Compatible with a wide range of detection systems**

- Works in both ELISA and Western applications
- Does not cross-react with rabbit antibodies
- Serum protein-free
- Biotin-free

**Shorter blocking times**

- "No-wait" blocking capability

**Superior signal:noise ratios in ELISA applications**

- Signal:noise ratios in the range of 10:1-20:1 have been realized with StartingBlock Blocking Buffer

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37538 | **StartingBlock (PBS) Blocking Buffer**<br>A protein-based blocker formulation in phosphate-buffered saline (pH 7.5) for use in ELISA and Western blotting applications. | 1L |
| 37542 | **StartingBlock (TBS) Blocking Buffer**<br>A protein-based blocker formulation in Tris-buffered saline (pH 7.5) for use in ELISA and Western blotting applications. | 1L |

StartingBlock Blocking Buffers are also available with an optimized amount of Tween-20 detergent to provide the lowest background.

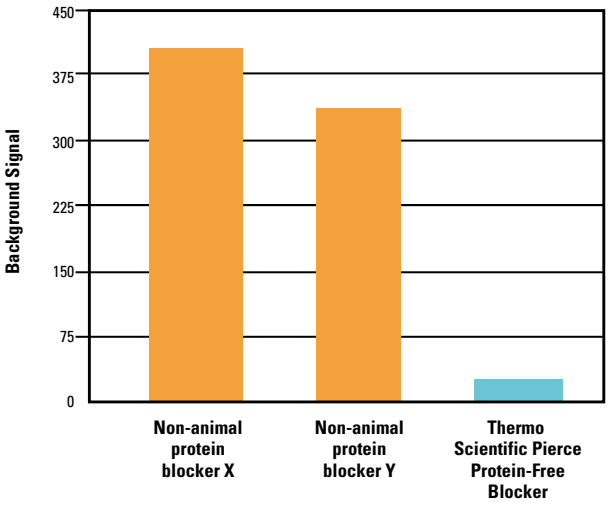
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37539 | **StartingBlock T20 (PBS) Blocking Buffer**<br>A protein-based blocker formulation in phosphate-buffered saline at pH 7.5 with 0.05% Tween-20 and Kathon® Antimicrobial Agent. | 1L |
| 37543 | **StartingBlock T20 (TBS) Blocking Buffer**<br>AA protein-based blocker formulation in Tris-buffered saline at pH 7.5 with 0.05% Tween-20 and Kathon Antimicrobial Agent. | 1L |

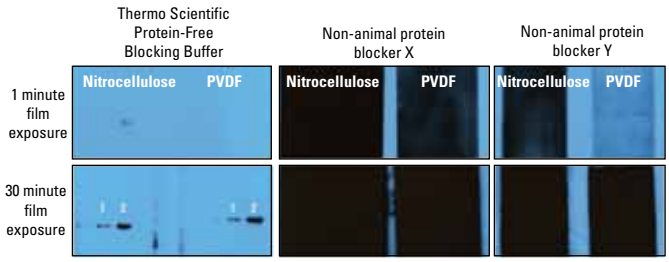
## Protein-Free Blocking Buffers

*Minimize background with protein free blockers.*

Traditional blocking buffers contain proteins that can cross-react with your system, resulting in thigh background and reduced signal. Use Thermo Scientific Pierce Protein-Free Blocking Buffers to eliminate or minimize cross-reactivity associated with protein-based blocking buffers in ELISA, Western Blotting, arrays and other immunodetection applications.




**Thermo Scientific Pierce Protein-Free Blocking Buffers exhibit less background than other blocking buffers in plate-based Thermo Scientific SearchLight Multiplex Arrays.** SearchLight Arrays were created by spotting up to 12 cytokine capture antibodies per well. The plates were then blocked with the indicated blocking buffers and the background for each well determined.




**Thermo Scientific Pierce Protein-Free Blocking Buffers efficiently blocks Western blotting membranes.** Jurkat Apoptotic Lysate (Lane 1: 0.25µg, Lane 2: 0:50µg) was separated in 4-20% Tris-glycine gels and transferred to nitrocellulose or PVDF membranes. The membranes were blocked for 1 hour at room temperature with the indicated blocking buffer, probed with mouse anti-PARP and detected by chemiluminescence with Thermo Scientific SuperSignal West Dura Extended Duration Substrate (Product # 34076) after incubation with the appropriate secondary antibody-HRP conjugate.

### Highlights:

- **Protein free; derived from non-animal, non-plant resources** – eliminates or minimizes cross reactivity associated with protein-based blocking buffers
- **Available with or without 0.05% Tween-20 detergent already added** – saves you time and money, eliminates errors, and minimizes the risk of contamination
- **Compatible with many detection systems** – can be used in Western blots, ELISA or arrays; no interference with avidin-biotin systems
- **High signal-to-noise values** – optimal sensitivity in customer applications
- **1X formulation** – ready to use

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37570 | **Pierce Protein-Free (TBS) Blocking Buffer** Proprietary formulation in Tris-buffered saline at pH 7.4 with Kathon Antimicrobial Agent | 1L |
| 37571 | **Pierce Protein-Free T20 (TBS) Blocking Buffer** Proprietary formulation in Tris-buffered saline at pH 7.4 with 0.05% Tween-20 and Kathon Antimicrobial Agent | 1L |
| 37572 | **Pierce Protein-Free (PBS) Blocking Buffer** Proprietary formulation in phosphate-buffered saline at pH 7.4 with Kathon Antimicrobial Agent | 1L |
| 37573 | **Pierce Protein-Free T20 (PBS) Blocking Buffer** Proprietary formulation in phosphate-buffered saline at pH 7.4 with 0.05% Tween-20 and Kathon Antimicrobial Agent | 1L |

## SuperBlock Blocking Buffers

*Guaranteed to be biotin-free.*

Our most popular blocking buffer, Thermo Scientific SuperBlock Blocking Buffer, now comes in both dry and liquid formats! Many researchers have discovered that our SuperBlock Blocking Buffer is the only blocking buffer needed for all of their applications.

### Highlights:

- Fast blocking – blocks ELISA plates in two minutes or membranes in five to 10 minutes
- Non-serum protein solution yields a very high signal:noise ratio
- Plates blocked with our SuperBlock Blocking Buffer can be stored dry for up to 12 months
- Liquid formulations available in PBS or TBS
- Biotin-free

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37515 | SuperBlock (PBS) Blocking Buffer | 1L |
| 37535 | SuperBlock (TBS) Blocking Buffer | 1L |

SuperBlock Blocking Buffers are also available with an optimized amount of Tween-20 detergent to provide the lowest background.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37516 | SuperBlock T20 (PBS) Blocking Buffer<br>(Contains 0.05% Tween-20) | 1L |
| 37536 | SuperBlock T20 (TBS) Blocking Buffer<br>(Contains 0.05% Tween-20) | 1L |

## SuperBlock Dry Blend (TBS) Blocking Buffers

*Delivers the ultimate in space-saving convenience.*

### Highlights:

- Delivers even more economy and stability
- Each pouch reconstitutes to form 200mL of SuperBlock Blocking Buffer in TBS
- Room-temperature storage; small packaging requires minimal shelf space


**References**

Ikeda, K., *et al.* (2003). *J. Biol. Chem.* **278**, 7725-7734.
Leclerc, G.J. and Barredo, J.C. (2001). *Clin. Cancer Res.* **7**, 942-951.
Subbarayan, V., *et al.* (2001). *Cancer Res.* **61**, 2720-276.
Walters, R.W., *et al.* (2002). *Cell* **100**, 789-799.


### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37545 | SuperBlock (TBS) Blocking Buffer Dry Blend Blocking Buffer<br>Each pouch yields 200mL when reconstituted. | 5 pouches |

## SEA BLOCK Blocking Buffer

*No mammalian proteins, reducing the risk of nonspecific interaction.*

### Highlights:

- Made from steelhead salmon serum
- Functions as a universal blocker
- Can be diluted up to 1:10 with buffer


**References**

Hypolite, J.A., *et al.* (2001). *Amer. J. Physiol. – Cell. Physiol.* **280**, C254-264.
Wang, L., *et al.* (2002). *J. Clin. Invest.* **110**, 1175-1184.


### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37527 | SEA BLOCK Blocking Buffer in PBS | 500mL |

## Blocker Casein

*Ready-to-use solution (1% w/v) of Hammersten grade casein for blocking nonspecific sites.*

### Highlights:

- Preformulated for ease of use
- An alternative when skim milk results in high background
- Thimerosal-free formulation

**References**
Nemzek, J.A., *et al.* (2000). *Amer. J. Physiol. – Lung Cell. Mol. Physiol.* **278**, L512-520.
Stuyver, L.J., *et al.* (2003). *Antimicrob. Agents Chemother.* **47**, 244-254.
Wolfman, J.C., *et al.* (2002). *Mol. Cell. Biol.* **22**, 1589-1606.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37532 | **Blocker Casein in TBS**<br>1% (w/v) Casein Hammersten Grade in TBS, Contains Kathon Antimicrobial Reagent as preservative, pH 7.4. | 1L |
| 37528 | **Blocker Casein in PBS**<br>1% (w/v) Casein Hammersten Grade in PBS, Contains Kathon Antimicrobial Reagent as preservative, pH 7.4. | 1L |

## Blocker BLOTTO

*Ready-to-use blocking buffers made of nonfat dry milk.*

### Highlights:

- Preformulated for ease of use
- Anti-foaming agent added
- Available in Tris-buffered saline
- Merthiolate-free formulation

**References**
Goretzki, L., *et al.* (2000). *J. Biol. Chem.* **275**, 28625-28633.
Abrams, E.T., *et al.* (2003). *J. Immunol.* **170**, 2759-2764.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37530 | **Blocker BLOTTO in TBS**<br>5% (w/v) nonfat powdered milk in TBS, 0.01% Anti-foam A, contains Kathon Antimicrobial Reagent as preservative, pH 7.4. | 1L |

## Blocker BSA

*For all blocking applications.*

### Highlights:

- 10% solutions of high-quality bovine serum albumin
- Concentrated formulation saves storage space
- No waiting for powder to dissolve with this ready-to-dilute liquid concentrate

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 37525 | **Blocker BSA in PBS (10X)** | 200mL |
| 37520 | **Blocker BSA in TBS (10X)** | 125mL |

## Surfact-Amps 20 Purified Detergent Solution

*Specially purified form of Tween-20 detergent.*

### Highlights:

- Guaranteed < 1 milliequivalent of peroxides and carbonyl in a 10% solution
- Enhances signal:background ratio

**Reference**
Ebong, S.J., *et al.* (2001). *Infect. Immun.* **69**, 2099-2106.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 28320 | **Surfact-Amps 20 Purified Detergent Solution** | 6 x 10mL |

## Nuclease-free Blocker Casein Blocking Buffer

*Please inquire for more details.*

### Thermo Scientific Blocking Buffers Application Chart

| Product # | Description | ELISA | Western blot | Dot blot | Immunohisto-chemistry | DNA/RNA Hybridizations |
|---|---|---|---|---|---|---|
| 37570 | Pierce Protein-Free (TBS) Blocking Buffer | ✓ | ✓ | | ✓ | |
| 37571 | Pierce Protein-Free T20 (TBS) Blocking Buffer | ✓ | ✓ | | ✓ | |
| 37572 | Pierce Protein-Free (PBS) Blocking Buffer | ✓ | ✓ | | ✓ | |
| 37573 | Pierce Protein-Free T20 (PBS) Blocking Buffer | ✓ | ✓ | | ✓ | |
| 37538 | StartingBlock (PBS) Blocking Buffer | ✓ | ✓ | ✓ | ✓ | |
| 37542 | StartingBlock (TBS) Blocking Buffer | ✓ | ✓ | ✓ | ✓ | |
| 37539 | StartingBlock T20 (PBS) Blocking Buffer | ✓ | ✓ | ✓ | ✓ | |
| 37543 | StartingBlock T20 (TBS) Blocking Buffer | ✓ | ✓ | ✓ | ✓ | |
| 37515 | SuperBlock Blocking Buffer in PBS | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37535 | SuperBlock Blocking Buffer in TBS | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37516 | SuperBlock T-20 PBS Blocking Buffer | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37536 | SuperBlock T-20 TBS Blocking Buffer | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37527 | SEA BLOCK Blocking Buffer | ✓ | ✓ | ✓ | | |
| 37520 | Blocker BSA in TBS | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37525 | Blocker BSA in PBS | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37532 | Blocker Casein in TBS | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37528 | Blocker Casein in PBS | ✓ | ✓ | ✓ | ✓ | ✓ |
| 37530 | Blocker BLOTTO in TBS | ✓ | ✓ | ✓ | ✓ | ✓ |

## Surfact-Amps Purified Detergent Solutions

*Nothing could be easier…nothing protects better!*

### Highlights:

- Disrupts nonspecific protein interactions
- Ampules are packed under nitrogen for maximum shelf life and are pre-scored for easy opening
- Gentle – non-denaturing at low concentrations
- Clear and pure 10% solutions – less than 1.0µeq/mL peroxides and carbonyl content
- Unparalleled convenience – solutions are purified, prediluted and sealed in ampules

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **Tween-20-Based Detergents** | | |
| **28320** | **Surfact-Amps 20** (Active Ingredient: Tween-20) | 6 x 10mL |
| **28328** | **Surfact-Amps 80** (Active Ingredient: Tween-80) | 6 x 10mL |
| **Triton-based Detergents** | | |
| **28314** | **Surfact-Amps X-100** (Active Ingredient: Triton X-100) | 6 x 10mL |
| **28332** | **Surfact-Amps X-114** (Active Ingredient: Triton X-114) | 6 x 10mL |
| **Nonidet-based Detergent** | | |
| **28324** | **Surfact-Amps NP-40** (Active Ingredient: Nonidet P-40) | 6 x 10mL |
| **Brij-based Detergents** | | |
| **28316** | **Surfact-Amps 35** (Active Ingredient: Brij-35) | 6 x 10mL |
| **28336** | **Surfact-Amps 58** (Active Ingredient: Brij-58) | 6 x 10mL |
| **20150** | **Brij-35, 30% Solution** | 950mL |

*Active ingredient is supplied as a purified 10% aqueous solution ampuled under nitrogen.*

## Surfact-Amps Detergent Sampler

*Convenient 10-sample package of detergents allows for testing and experimentation.*

### Ordering Information

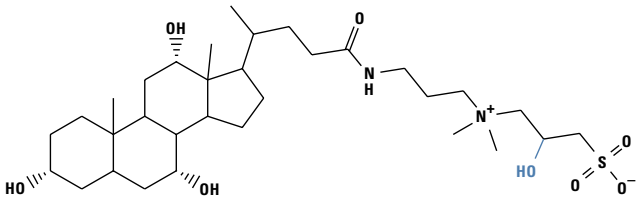
| Product # | Description | Pkg. Size |
|---|---|---|
| **28340** | **Surfact-Amps Detergent Sampler** | Kit |
| | Contains: Surfact-Amps Purified Detergents | |
| | Surfact-Amps X-100 | 10mL |
| | Surfact-Amps 35 | 10mL |
| | Surfact-Amps 20 | 10mL |
| | Surfact-Amps NP-40 | 10mL |
| | Surfact-Amps 80 | 10mL |
| | Surfact-Amps X-114 | 10mL |
| | Surfact-Amps 58 | 10mL |
| | Octyl β-Glucoside | 100mg |
| | Octyl β-Thioglucopyranoside | 100mg |
| | CHAPS | 100mg |

## CHAPS & CHAPSO

*Zwitterionic detergents that are ideal for protecting the native state of proteins.*

### Highlights:

- Non-denaturing
- Able to disrupt nonspecific protein interactions
- Less protein aggregation than nonionic detergents
- Electrically neutral.




**CHAPS**
**M.W. 614.88**

**CHAPSO**
**M.W. 630.88**

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **28300** | **CHAPS** (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate) | 5g |
| **28299** | **CHAPS** | 100g |
| **28304** | **CHAPSO** (3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate) | 5g |

## Washing the Microplate

Many immunoassay procedures include a series of incubations with different immunochemical reagents separated by wash steps. Washing steps are necessary to remove unbound reagents and reduce background, thereby increasing the signal:noise ratio. Insufficient washing will allow high background, while excessive washing may result in decreased sensitivity caused by elution of the antibody and/or antigen from the well. A variety of buffers may be used. Occasionally, washing is performed in a physiologic buffer such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) without any additives. More commonly, a detergent such as 0.05% Tween-20 (Product # 28320) is added to the buffer to help remove nonspecifically bound material. Another common technique is to use a dilute solution of the blocking buffer along with some added detergent. Including the blocking agent and adding a detergent in wash buffers helps to minimize background in the assay. For best results, use high-purity detergents, such as Thermo Scientific Surfact-Amps Detergents.

### Thermo Scientific BupH Dry Buffers

The most advanced, versatile, time-saving buffer product line available.

**The ultimate in convenience**

1. Reach for the sealed foil pack sitting conveniently on your bench top.
2. Open, pour into beaker and add water.
3. The fresh buffer is ready to use in practical aliquots so there's no waste.

**The ultimate in versatility**

1. Routine buffers are designed for use in Western blotting, dialysis, crosslinking, ELISAs, immunohistochemistry, protein plate-coating, biotinylation and other applications.
2. Using one buffer source maintains consistency.

**The ultimate in integrity**

1. Our BupH Buffers are protected from contamination and are fresh every time.
2. Perform applications with confidence in high quality buffers.
3. "Test-assured" with our commitment to quality management standards.

**The ultimate in time savings**

1. Making routine buffers is no longer time-consuming.
2. No component measurement, pH adjustment, quality validation, preparation tracking or refrigeration hassles.
3. Move forward with your work by eliminating re-tests caused by buffer problems.

## BupH Phosphate Buffered Saline Packs

*Great wash buffer!*

Each pack yields 500mL of 0.1M phosphate, 0.15 M NaCl, pH 7.0 when dissolved in 500mL deionized water (20 liters total).

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 28372 | BupH Phosphate-Buffered Saline Packs | 40 pack |

## BupH Tris Buffered Saline Packs

*Great wash buffer!*

Each pack yields 500mL of 25mM Tris, 0.15 M NaCl, pH 7.2 when dissolved in 500mL deionized water (10 pack makes 5 liters total; 40 pack makes 20 liters total).

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 28380 | BupH Tris-Glycine Buffer Packs | 40 pack |
| 28376 | BupH Tris-Buffered Saline Packs | 40 pack |
| 28379 | BupH Tris-Buffered Saline Packs | 10 pack |

## Surfact-Amps 20 Purified Detergent Solution

*Specially purified form of Tween-20.*

### Highlights:

- Can be added to PBS or TBS wash buffers to improve performance
- Guaranteed < 1 milliequivalent of peroxides and carbonyl in a 10% solution
- Enhances signal:background ratio

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 28320 | Surfact-Amps 20 | 6 x 10mL |

# Detection Probes

## Selecting Antibodies

**The choice of a primary antibody will depend on the antigen to be detected and what antibodies are available to that antigen.** A large number of primary antibodies are available commercially and can be identified quickly by searching sites such as *www.antibodyresource.com*. Alternatively, a primary antibody may be made to recognize the antigen of interest. Both polyclonal and monoclonal antibodies work well for ELISA. Polyclonal antibodies are less expensive and less time-consuming to produce and often have a high affinity for the antigen. Monoclonal antibodies are valued for their specificity, purity and consistency that result in lower background. Crude antibody preparations such as serum or ascites fluid are sometimes used for ELISA, but the impurities present may increase background. To obtain antibodies with the greatest specificity, they can be affinity-purified using the immobilized antigen.

A wide variety of labeled secondary antibodies can be used for ELISA detection. The choice of secondary antibody depends upon the species of animal in which the primary antibody was raised (the host species). For example, if the primary antibody is a mouse monoclonal antibody, the secondary antibody must be an anti-mouse antibody obtained from a host other than the mouse. The host species of the secondary antibody often will not affect the experiment. However, secondary antibodies are available from several different host species and, if a secondary antibody causes high background in a particular assay, another host species may improve results. Another option to reduce background is to use a secondary antibody that has been pre-adsorbed to serum proteins from other species. This pre-adsorption process removes antibodies that have the potential to cross-react with serum proteins, including antibodies. To expedite the process of choosing the appropriate secondary antibody, use the Secondary Antibody Selection Guide located on our website.

Antibodies for ELISA are typically used as diluted 1/100-1/500,000 beginning from a 1mg/mL stock solution. The optimal dilution of a given antibody with a particular detection system must be determined experimentally. More sensitive detection systems require less antibody, which can result in substantial savings on antibody costs and allow a limited supply of antibody to be used for more experiments. It also produces a side benefit of reduced background because the limited amount of antibody is specific for the target with the highest affinity. Antibody dilutions are typically made in the wash buffer containing a blocking agent. The presence of a small amount of blocking agent and detergent in the antibody diluent often helps to minimize background.

We offer a wide variety of Thermo Scientific Pierce Labeled Secondary Antibodies for use in ELISA. The labels include biotin, fluorescein, Thermo Scientific DyLight dyes, rhodamine, horseradish peroxidase and alkaline phosphatase. For the complete list of labeled secondary antibodies please refer to pages 34-35.

## Antibodies

*Over 30,000 antibodies in 42 research areas.*

Thermo Scientific Pierce Antibodies are developed for a wide variety of application needs. Our website enables you to easily search by protein target and then filter by the specific assays you are interested in. All of our antibodies are validated in the stated applications and are guaranteed to perform.

### Applications

- Agglutination
- Competition Assay
- ChIP Assay
- Cytotoxicity Assay
- Control
- ELISA
- Electron Microscopy
- FACS
- Functional Assay
- Gel Shift
- Hemagglutination Assay
- Inhibition Assay
- Immunocytochemistry
- Immunodiffusion
- Immunofluorescence
- Immunohistochemistry
- Immunohistochemistry (Frozen)
- Immunohistochemistry (Paraffin)
- Immunohistochemistry (Paraffin, Frozen)
- Infection
- Immunoprecipitation
- Immunoradiometric Assay
- Radioimmune Assay
- Western Blot

## Custom Antibody Services

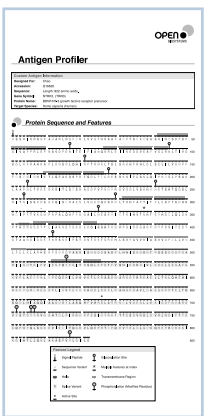
*Design and produce better custom antibodies.*

The Thermo Scientific Open Biosystems Custom Antibody Development Service leverages our experience in making more than 18,500 antibodies to peptides and recombinant proteins. Our proprietary antigen design tools, including the Thermo Scientific Antigen Profiler Software (below), and targeted antigen display produces more robust antibodies that perform better in your targeted assays.

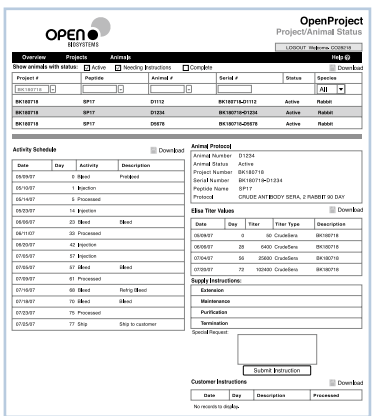
When you initiate a custom antibody project with us we provide you access to our online project management tool. This secure account gives you easy access to project information and allows you to provide specific instructions for your projects.

**For more information on Thermo Scientific Open Biosystems Custom Antibody Services, please visit www.thermoscientific.com/Custom-Abs.**

**A.**




**B.**




**Thermo Scientific Antigen Profiler Software provides the tools to develop better antibodies.** The online software allows you to examine comparative antigenicity based on *in vivo* titer data from over 13,000 peptide antibodies and to identify important structural motifs to produce high specificity antibodies. The software also includes a peptide tutorial to aid in synthesizing soluble peptides and curated BLAST analysis for specificity determination. A sample report **(A.)** and online project tool **(B.)** are shown here.

## Selecting Antibody Labels

The choice of secondary antibody also depends upon the type of label that is desired. Many different labels can be conjugated to antibodies. Radioisotopes were used extensively in the past, but they are expensive, have a short shelf life, offer no improvement in signal:noise ratio and require special handling. Alternative labels are biotin, fluorophores and enzymes. The use of fluorophores requires fewer steps; however, special equipment is needed to view the fluorescence. Also, a photograph must be taken if a permanent record of the results is desired. Enzymatic labels are used most commonly and provide excellent results.

Alkaline phosphatase (AP) and horseradish peroxidase (HRP) are the two enzymes that are used extensively as labels for protein detection. An array of chromogenic, fluorogenic and chemiluminescent substrates is available for use with either enzyme. For a detailed comparison of these two enzymes, see Table 2.

AP, a 140kDa protein that is generally isolated from calf intestine, catalyzes the hydrolysis of phosphate groups from a substrate molecule, resulting in a colored or fluorescent product or the release of light as a byproduct. AP has optimal enzymatic activity at a basic pH (pH 8-10) and can be inhibited by cyanides, arsenate, inorganic phosphate and divalent cation chelators, such as EDTA. As a label for ELISA, AP offers a distinct advantage over other enzymes. Because its reaction rate remains linear, detection sensitivity can be improved by simply allowing a reaction to proceed for a longer time period.

HRP is a 40kDa protein that catalyzes the oxidation of substrates by hydrogen peroxide, resulting in a colored or fluorescent product or the release of light as a byproduct. HRP functions optimally at a near-neutral pH and can be inhibited by cyanides, sulfides and azides. Antibody-HRP conjugates are superior to antibody-AP conjugates with respect to the specific activities of both the enzyme and antibody. In addition, its high turnover rate, good stability, low cost and wide availability of substrates make HRP the enzyme of choice for most applications.

**Table 2. Comparison of horseradish peroxidase and alkaline phosphatase enzymes.**

| | Horseradish Peroxidase | Alkaline Phosphatase |
|---|---|---|
| **Size** | 40kDa | 140kDa |
| **Price** | Relatively Inexpensive | Relatively Expensive |
| **Stability (Storage)** | Stable at < 0°C | Unstable at < 0°C |
| **Number of Substrates** | Many | Few |
| **Kinetics** | Rapid | Slower |
| **ph optimum** | 5-7 | 8-10 |

## Antibody Conjugates and Signal Detection

After blocking, the primary antibody is incubated in the microplate, allowing it to bind to the antigen. The time required for this step typically ranges from 1 hour at room temperature to overnight at 4°C, depending upon the affinity of the antibody for its antigen. The primary antibody may have been labeled to create a direct ELISA. Care must be taken that the antibody conjugate is stored properly and that it has been diluted in the blocking buffer with or without Tween-20 detergent when performing the ELISA. Peroxidase-conjugated antibodies may be stored in Thermo Scientific Pierce Peroxidase Conjugate Stabilizer (Product # 37548) to a 1:1,000 dilution or to the desired assay dilution range. This stabilizer prevents the loss of enzyme activity over time, removes the need to aliquot and freeze conjugates, and allows the antibody conjugate to be stored at 4°C.

An alternative to the direct method is to use a labeled secondary antibody that recognizes the primary antibody species. Many secondary antibody conjugates are commercially available with common enzyme labels such as AP or HRP. If the primary antibody has a biotin tag, then the secondary reagent would be a streptavidin or Thermo Scientific NeutrAvidin Enzyme Conjugate, with an alternative being the avidin-biotin complex (ABC) with a biotinylated enzyme (Figure 1, page 5). Selecting the appropriate protocol for an ELISA depends upon the availability of the primary antibody, the sensitivity level desired and the nature of the sample being tested. We provide a variety of secondary antibodies, NeutrAvidin Protein and streptavidin conjugates to choose from in designing an ELISA with low background and high reproducibility.

Enzyme-antibody conjugates often yield best results when diluted in the same material that was used for blocking with the addition of high-quality Tween-20 detergent to a concentration of 0.05%. Figure 6 compares SuperBlock Blocking Buffer with Tween-20 detergent to other proteins for use as conjugate diluents. It has Tween-20 detergent, as a component of the primary and secondary antibody diluents, often provides increased signal:noise ratios. Surfact-Amps 20 (Product # 28320) is a highly purified Tween-20 detergent solution, free of peroxides and carbonyls that can cause ELISA artifacts.

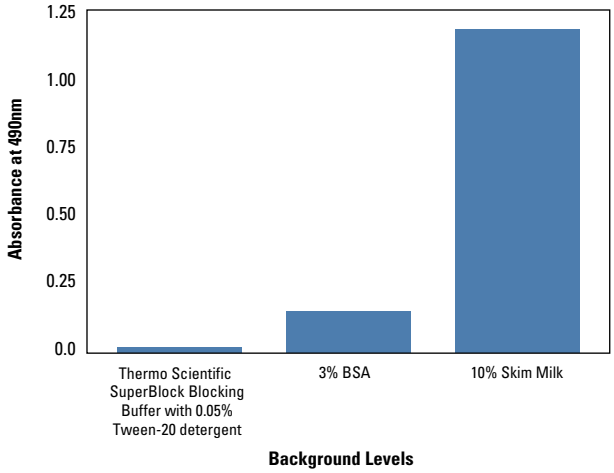



**Figure 6. Thermo Scientific SuperBlock Blocking Buffers vs. other proteins for use as conjugate diluents.** SuperBlock Blocking Buffers with 0.05% Tween-20 detergent is the superior diluent.

The final stage in all ELISA systems is a detection step in which a substrate is introduced, and the bound enzyme (indicating the presence of analyte) converts that substrate to a detectable product. The intensity of the signal produced when the substrate is added should correlate to the concentration of the primary antibody and the respective antigen. Enzyme-labeled reagents may be detected using chromogenic, chemifluorescent or chemiluminescent substrates. When performing ELISAs, a soluble substrate is used to generate a signal in solution. Chemiluminescent signals emit light that is measured by a luminometer and fluorescent substrates require an excitation for light to be emitted and detected by a fluorometer (Table 3).

**Table 3. Choosing a detection method.**

| | Colorimetric Substrates | Chemifluorescent Substrates | Chemiluminescent Substrates |
|---|---|---|---|
| **ELISA** | • Medium/low sensitivity<br>• Generally less expensive<br>• Many substrates available<br>• Slow signal generation<br>• Enzyme catalyzed quickly<br>• Small linear range/poor low-end linearity<br>• Flexible (stopped, nonstopped and kinetic assays) | • High sensitivity<br>• Generally more expensive<br>• Few substrates available<br>• Rapid signal generation<br>• Enzyme activity maintained<br>• Large linear range/enhanced low-end linearity<br>• Flexible (stopped, nonstopped and kinetic assays) | • High sensitivity<br>• Generally more expensive<br>• Few substrates available<br>• Rapid signal generation<br>• Enzyme catalyzed quickly<br>• Large linear range/enhanced low end linearity<br>• Nonflexible |
| **Detection Method** | Spectrophotometer provides quantifiable results | Fluorometer provides quantifiable results | Luminometer provides quantifiable results |

## Affinity-Purified Secondary Antibodies

Thermo Scientific Pierce Affinity-Purified Antibodies are available unconjugated or conjugated with biotin, alkaline phosphatase, horseradish peroxidase, fluorescein, rhodamine or DyLight Dyes. $F(ab')_2$ fragments of antibodies to immunoglobulins are also available in unconjugated or conjugated forms. These $F(ab')_2$ fragments of antibodies are especially useful in assays in which binding between the Fc portions of antibodies and Fc receptor-bearing cells must be eliminated.

Our Polyclonal Antibodies are purified by immunoaffinity chromatography using antigens coupled to agarose gels. Affinity purification helps to eliminate nonspecific antibodies, resulting in an increase in sensitivity and specificity, with a decrease in background. The purification process involves an elution procedure, yielding antibodies with high avidity. These antibodies exhibit both maximal binding to antigens and minimal cross-reactivity to other molecules. Conjugated antibodies are affinity-purified before conjugation.

Selected Pierce Antibodies have been further purified by passing them through immobilized serum proteins from other species. This additional processing minimizes cross-reactivities to other species' serum proteins and is indicated by "min x Species Sr Prot." The abbreviations for the individual species are listed in the table below.

**Key to abbreviations for individual species.**

| | | | |
|---|---|---|---|
| Bv = Bovine | Gu = Guinea Pig | Hs = Horse | Rt = Rat |
| Ch = Chicken | Ha = Hamster | Ms = Mouse | Sh = Sheep |
| Gt = Goat | Hn = Human | Rb = Rabbit | Sw = Swine |

Pierce Polyclonal Conjugated Antibodies contain bovine serum albumin as a stabilizer. The following table lists the typical working dilutions for the conjugated antibodies when performing ELISAs.

**Typical dilution ranges recommended for Thermo Scientific Pierce Polyclonal Conjugated Antibodies for ELISAs.**

| Conjugate | ELISA |
|---|---|
| Alkaline Phosphatase | 1:5000-1:50,000 |
| Peroxidase | 1:5000-1:200,000<br>(for SuperSignal ELISA Products) |

### Storing Enzyme Conjugates

A variety of Thermo Scientific reagents are available to help preserve enzyme conjugate activity. Typically, conjugates are aliquoted in 50-100µL increments using purified ethylene glycol (Product # 29810) as a preservative for –20°C storage. Conjugates can maintain activity for up to two years. An alternative to aliquoting is to use Thermo Scientific Pierce Peroxidase Conjugate Stabilizer (Product # 31503), diluting the conjugate 1:1 in the stabilizer and storing it at –20°C for up to one year as a stock solution. Pierce Peroxidase Stabilizer/Diluents (Product #s 37548 and 37552) allow peroxidase conjugates to be reconstituted and stored at 4°C as a 1:1000 dilution or a 1:100,000 dilution stock solution.

### Conjugate Stabilizers

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **37548** | Pierce Peroxidase Conjugate Stabilizer/Diluent | 200mL |
| **37552** | Pierce Peroxidase Conjugate Stabilizer/Diluent | 1L |
| **31503** | Pierce Peroxidase Conjugate Stabilizer | 25mL |
| **29810** | Ethylene Glycol<br>(50% aqueous solution) | 200mL |

## Ordering Information

| Specificity | Source | Unconjugated | Biotin | Peroxidase | Alk. Phos. | Fluorescein |
|---|---|---|---|---|---|---|
| Chicken IgY (H+L) | Rabbit | 31104 | 31720 | 31401 | | 31501 |
| Goat IgG (H+L) (min x HnMsRb Sr Prot)† | Mouse | 31107 | 31730 | 31400 | | 31512 |
| Goat IgG (H+L) | Rabbit | 31105 | 31732 | 31402 | 31300 | 31509 |
| Goat IgG [F(ab')$_2$] | Rabbit | 31214 | 31753 | 31403 | | 31553 |
| Goat IgG (Fc) | Rabbit | 31133 | | 31433 | 31337 | 31533 |
| Goat IgG (H+L) (min x Hn Sr Prot)† | Rabbit F(ab´)$_2$ | 31109 | | | 31302 | |
| Hamster IgG (H+L) | Goat | 31115 | 31750 | | | |
| Hamster IgG (H+L) | Rabbit | 31120 | | | | 31587 |
| Horse IgG (H+L) | Goat | | 31760 | | | |
| Human IgG (H+L) | Goat | 31130 | 31770 | 31410 | 31310 | 31529 |
| Human IgG Gamma Chain Specific | Goat | 31118 | | | | |
| Human IgG (H+L) (min x BvHsMs Sr Prot)† | Goat | 31119 | 31774 | 31412 | | 31531 |
| Human IgG [F(ab')$_2$] | Goat | 31122 | | 31482 | 31312 | 31628 |
| Human IgG [F(ab')$_2$] (min x BvHsMs Sr Prot)† | Goat | 31132 | | 31414 | | |
| Human IgG (Fc) (min x BvHsMs Sr Prot)† | Goat | 31125 | | 31413 | | |
| Human IgM (Fc5µ) | Goat | 31136 | | 31415 | | 31575 |
| Human IgM (µ) | Goat | 31124 | 31778 | | | |
| Human IgA (α) | Goat | 31140 | | 31417 | 31314 | 31577 |
| Human IgA + IgG + IgM (H+L) | Goat | 31128 | 31782 | 31418 | 31316 | |
| Human Kappa Chain | Goat | 31129 | 31780 | | | |
| Human Lambda Chain | Goat | 31131 | | | | |
| Human IgG (H+L) (min x Ms Sr Prot)† | Mouse | 31135 | | 31420 | | |
| Human IgG (H+L) (min x BvHsMs Sr Prot)† | Mouse | 31137 | 31784 | | | |
| Human IgG (H+L) | Rabbit | 31143 | 31786 | | | |
| Human IgG (Fc) | Rabbit | 31142 | 31789 | 31423 | 31318 | 31535 |
| Human IgG (Fc) | Goat F(ab´)$_2$ | 31163 | | | | |
| Human IgG (H+L) | Goat F(ab´)$_2$ | 31165 | | | | |
| Human IgA + IgG + IgM (H+L) | Goat F(ab´)$_2$ | | | | | 31539 |
| Mouse IgA (α) (min x Hn Sr Prot)† | Goat | 31169 | | | | |
| Mouse IgA + IgG + IgM (H+L) | Goat | 31171 | | | | |
| Mouse IgG (H+L) | Goat | 31160 | 31800 | 31430†† | 31320 | 31569 |
| Mouse IgG (H+L), Highly Cross-adsorbed | Goat | | | | | |
| Mouse IgG (H+L) (min x BvHnHs Sr Prot)† | Goat | 31164 | 31802 | 31432 | 31322 | 31541 |
| Mouse IgG [F(ab')$_2$] | Goat | 31166 | 31803 | 31436 | 31324 | 31543 |
| Mouse IgG (Fc) | Goat | 31168 | 31805 | 31437 | 31325 | 31547 |
| Mouse IgG (Fc) (min x BvHnHs Sr Prot)† | Goat | 31170 | | 31439 | 31327 | 31632 |
| Mouse IgM (µ) | Goat | 31172 | 31804 | 31440 | 31326 | 31992 |
| Mouse IgG + IgM (H+L) | Goat | 31182 | 31807 | 31444 | 31328 | |
| Mouse IgG + IgM (H+L) (min x BvHnHs Sr Prot)† | Goat | | | 31446 | 31330 | |
| Mouse IgG (Fcγ) (subclasses 1+2a+2b+3) (min x BvHnRb Sr Prot)† | Goat | 31232 | | | | 31630 |
| Mouse IgG (Fcγ) subclass 1 specific (min x BvHnRb Sr Prot)† | Goat | 31236 | | | | |
| Mouse IgG (Fcγ) subclass 2a specific (min x BvHnRb Sr Prot)† | Goat | 31237 | | | | 31634 |
| Mouse IgG (H+L) | Horse | 31181 | 31806 | | | |
| Mouse IgG (H+L) | Rabbit | 31188 | | 31450 | 31329 | 31561 |
| Mouse IgG (H+L) (min x Hn Sr Prot)† | Rabbit | 31190 | | 31452 | 31334 | |
| Mouse IgG [F(ab')$_2$] | Rabbit | 31192 | | 31451 | 31331 | 31559 |
| Mouse IgG (Fc) | Rabbit | 31194 | 31813 | 31455 | 31332 | 31555 |
| Mouse IgM (µ) | Rabbit | 31196 | | 31456 | 31333 | 31557 |
| Mouse IgG + IgM (H+L) | Rabbit | 31198 | | 31457 | 31335 | 31558 |
| Mouse IgG (H+L) (min x BvHnHs Sr Prot)† | Goat F(ab´)$_2$ | 31185 | | 31438 | | 31565 |
| Mouse IgM (µ) | Goat F(ab´)$_2$ | 31178 | | | | |
| Mouse IgM (µ) (min x BvHnHs Sr Prot)† | Goat F(ab´)$_2$ | 31186 | | | | |
| Mouse IgG + IgM (H+L) (min x BvHnHs Sr Prot)† | Goat F(ab´)$_2$ | | | 31448 | | |
| Rabbit IgG (H+L) (min x BvChGtGuHaHnHsMsRtSh Sr Prot)† | Donkey | 31238 | 31821 | 31458 | 31345 | 31568 |
| Rabbit IgG (H+L) | Goat | 31210 | 31820 | 31460†† | 31340 | 31635 |
| Rabbit IgG (H+L), Highly Cross-Adsorbed | Goat | | | | | |
| Rabbit IgG (H+L) (min x Hn Sr Prot)† | Goat | 31212 | 31822 | 31462 | 31342 | 31583 |
| Rabbit IgG [F(ab')$_2$] | Goat | 31234 | 31823 | 31461 | 31343 | 31573 |
| Rabbit IgG (Fc) | Goat | 31216 | | 31463 | 31341 | |
| Rabbit IgG (H+L) (min x GtHnMsSh Sr Prot)† | Mouse | 31213 | 31824 | 31464 | | 31584 |
| Rabbit IgG (H+L) | Goat F(ab´)$_2$ | | | | | 31579 |
| Rabbit IgG (H+L) (min x HnMsRt Sr Prot)† | Goat F(ab´)$_2$ | 31239 | | | | 31636 |
| Rat IgG (H+L) | Goat | 31220 | 31830 | 31470 | 31350 | 31629 |
| Rat IgG [F(ab')$_2$] | Goat | | | 31474 | | |
| Rat IgG (Fc) | Goat | 31226 | | 31475 | | 31621 |
| Rat IgM (µ) | Goat | | 31832 | 31476 | 31354 | 31631 |
| Rat IgG (H+L) | Rabbit | 31218 | 31834 | | | |
| Rat IgG (H+L) (min x Ms Sr Prot)† | Rabbit | 31219 | | | | |
| Sheep IgG (H+L) | Rabbit | 31240 | 31840 | 31480 | 31360 | 31627 |
| Streptavidin | Recombinant | 21125 | | 21127 | 21323 | 21224 |
| NeutrAvidin Protein | Hen Egg | 31000 | | 31001 | 31002 | 31006 |

*† See Table on page 33 for the Key to Abbreviations.*
*†† Stabilized, pre-diluted format also available; see our web site.*

*For pricing in the U.S., visit www.thermoscientific.com/pierce.*
*Outside the U.S., please contact your local branch or distributor.*

Product #

| Rhodamine | Texas Red | DyLight 350 | DyLight 405 | DyLight 488 | DyLight 594 | DyLight 633 | DyLight 680 | DyLight 800 |
|---|---|---|---|---|---|---|---|---|
| 31620 | 31940 | | | | | | | |
| 31650 | 31492 | | | | | | | |
| 31656 | 31943 | | | | | | | |
| 31683 | 31944 | | | | | | | |
| 31660 | 31498 | 62271 | | 35502 | 35510 | | 35518 | 35521 |
| | | 62273 | 35500 | 35503 | 35511 | 35513 | 35519 | |
| 31661 | 31500 | | | | | | | |
| 31663 | | | | | | | | |
| 31665 | 31610 | | | | | | | |
| 31685 | 31504 | | | | | | | |
| 31670 | 31506 | 62270 | | 35552 | 35560 | | 35568 | 35571 |
| | | 62272 | 35550 | 35553 | 35561 | 35563 | 35569 | |
| 31686 | 31507 | | | | | | | |
| 31680 | 31508 | | | | | | | |
| 21724 | 21726 | | 21881 | 21832 | 21842 | 21844 | 21848 | 21851 |
| | | | 22831 | 22832 | 22842 | 22844 | 22848 | 22853 |

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 31148 | Human IgA, Serum | 2mg |
| 31154 | Human IgG, Whole Molecule | 10mg |
| 31146 | Human IgM (myeloma), Whole Molecule | 2mg |
| 31505 | Mouse IgG, Whole Molecule - Fluorescein Conjugated | 1mg |
| 31203 | Mouse IgG, F(ab')$_2$ Fragment | 2mg |
| 31205 | Mouse IgG, Fc Fragment | 1mg |
| 31453 | Mouse Transferrin - Peroxidase Conjugated | 1mg |
| 31235 | Rabbit IgG, Whole Molecule | 10mg |
| 31233 | Rat IgG, Whole Molecule | 10mg |
| 31243 | Sheep IgG, Whole Molecule | 25mg |

## Fluorescent-labeled Antibody Conjugates

### Thermo Scientific DyLight Conjugates

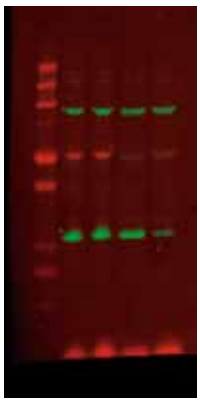
*Bright new alternatives to Alexa Fluor, CyDye and LI-COR Fluorescent Dyes.*

DyLight Dyes have absorption spectra ranging from 350nm to 770nm (Table 4) and match the principal output wavelengths of common fluorescence instrumentation. They exhibit higher fluorescence intensity and photostability than Alexa Fluor, CyDye and LI-COR Dyes in many applications and remain highly fluorescent over a broad pH range (pH 4-9). Additionally, DyLight Dye water solubility allows a high dye-to-protein ratio without precipitation during conjugation.

### Highlights:

- Available conjugated to commonly used secondary antibodies, streptavidin and NeutrAvidin Protein; conjugated using a molar ratio (dye:protein) optimized to provide excellent fluorescent intensity
- Stable for 1 year at 4°C
- Antibody conjugates are affinity-purified to minimize cross-reactivity
- Superior photostability
- pH-insensitive (pH 4-9)
- High water solubility
- Compatible with common fluorescence instrumentation

**Western Blotting**

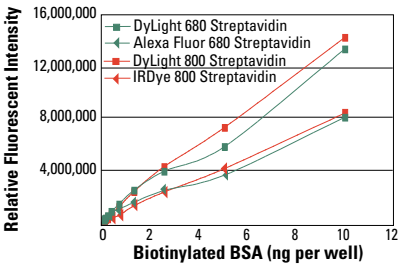



**Two-color infrared Western blot detection of p53 and cyclophilin B knockdown using Thermo Scientific DyLight 680- and Thermo Scientific DyLight 800-labeled secondary antibodies.** Protein lysate from transfected A549 cells was separated using SDS-PAGE and transferred to PVDF membrane. **Lane 1:** MW marker, **Lane 2:** mock transfected sample, **Lane 3:** negative control siRNA, **Lane 4:** siRNA targeted against p53 and **Lane 5:** siRNA targeted against cyclophilin. The membranes were imaged with the Odyssey Infrared Imaging System using the 700 and 800 channels.

**Table 4. Spectral properties of Thermo Scientific DyLight Fluorescent Dyes.**

| Emission | Color | Thermo Scientific DyLight Dye | Ex/Em* | ε† | Spectrally Similar Dyes |
|---|---|---|---|---|---|
| Blue | | 350 | 353/432 | 15,000 | Alexa Fluor 350 Dye |
| | | 405 | 400/420 | 30,000 | Alexa Fluor 405 and Cascade Blue® Dyes |
| Green | | 488 | 493/518 | 70,000 | Alexa Fluor 488, fluorescein and FITC Dyes |
| Red | | 594 | 593/618 | 80,000 | Alexa Fluor 594 and Texas Red® Dyes |
| | | 633 | 638/658 | 170,000 | Alexa Fluor 633 Dye |
| Near Infrared | | 680 | 692/712 | 140,000 | Alexa Fluor 680 and Cy5.5 Dyes |
| | | 800 | 777/790 | 270,000 | IRDye® 800 Dye |

**Excitation and emission maxima in nanometers (± 4nm).*
*†Molar extinction coefficient ($M^{-1}cm^{-1}$).*




**Thermo Scientific DyLight 680 and Thermo Scientific DyLight 800 Streptavidin Conjugates are brighter than Alexa Fluor 680 or IRDye 800 Conjugates in microplate-based assays.** Microplates were coated with biotinylated BSA at the indicated concentrations. Conjugates were diluted to 10µg/mL in PBS and 100µL was applied to each plate. Fluorescent intensity was measured with the Odyssey Infrared Imaging System using the 700 and 800 channels.

## Ordering Information

**Conjugates:** *Package size for these items is 1mg at 1mg/mL.*

| | Product # | | | | | | |
|---|---|---|---|---|---|---|---|
| **Description** | **DyLight 350 Dye** | **DyLight 405 Dye** | **DyLight 488 Dye** | **DyLight 594 Dye** | **DyLight 633 Dye** | **DyLight 680 Dye** | **DyLight 800 Dye** |
| Goat Anti-Mouse IgG (H+L) | 62271 | | 35502 | 35510 | | 35518 | 35521 |
| Goat Anti-Mouse IgG Highly Cross-Adsorbed | 62273 | 35500 | 35503 | 35511 | 35513 | 35519 | |
| Goat Anti-Rabbit IgG (H+L) | 62270 | | 35552 | 35560 | | 35568 | 35571 |
| Goat Anti-Rabbit IgG Highly Cross-Adsorbed | 62272 | 35550 | 35553 | 35561 | 35563 | 35569 | |
| Streptavidin | | 21831 | 21832 | 21842 | 21844 | 21848 | 21851 |
| NeutrAvidin Biotin-Binding Protein | | 22831 | 22832 | 22842 | 22488 | 22848 | 22853 |

## Antibody Binding Proteins

### Protein A

*Binds specifically to the Fc region of immunoglobulin molecules, especially IgG.*

**Highlights:**

- Isolated from native *Staphylococcus aureus* (MW = 42K)
- Contains four IgG-binding sites

**Ordering Information**

| Product # | Description | Pkg. Size |
|---|---|---|
| 21181 | Protein A | 5mg |
| 29989 | Biotinylated Protein A | 1mg |

### Protein A, Recombinant

*No enterotoxins present, as there may be from* Staphylococcus-*derived Protein A.*

**Highlights:**

- Harvested from a nonpathogenic form of *Bacillus*, which has been genetically designed to manufacture and secrete carboxy terminus truncated (MW ~ 44.6K) recombinant Protein A

**Ordering Information**

| Product # | Description | Pkg. Size |
|---|---|---|
| 21184 | Purified Protein A | 5mg |
| 32400 | Pierce Purified Recombinant Protein A, Peroxidase Conjugated | 1mg |

### Protein G, Recombinant

*Useful for a variety of immunological and biochemical techniques.*

**Highlights:**

- Protein G is a bacterial cell wall protein isolated from group G *Streptococci* (MW = 22K)
- Binds to most mammalian immunoglobulins through their Fc regions
- Albumin and cell surface binding sites have been removed from this recombinant form to reduce nonspecific binding when Protein G is used to purify, identify or locate immunoglobulins
- Useful for separating albumin from crude human or mouse IgG samples
- Binds with greater affinity to most mammalian immunoglobulins than Protein A, including human $IgG_3$ and rat $IgG_{2a}$
- Does not bind to human IgM, IgD and IgA

**Ordering Information**

| Product # | Description | Pkg. Size |
|---|---|---|
| 21193 | Pierce Purified Recombinant Protein G | 5mg |
| 29988 | Biotinylated Protein G | 0.5mg |
| 31499 | Protein G, Peroxidase Conjugated | 0.5mg |

### Protein A/G, Recombinant

*Produced by gene fusion of the Fc binding domains of Protein A and Protein G.*

**Highlights:**

- Protein A/G is a 50,449 dalton protein containing 442 amino acids, 43 of which are lysines
- Binds well to immunoglobulins over a broad pH range (pH 4-9)
- Contains four Protein A Fc binding domains and two Protein G Fc binding domains
- Binds all IgG subclasses of mouse immunoglobulins, making it an excellent tool for purification and detection of mouse monoclonal antibodies

**Ordering Information**

| Product # | Description | Pkg. Size |
|---|---|---|
| 21186 | Pierce Purified Recombinant Protein A/G | 5mg |
| 32391 | Protein A/G, Alkaline Phosphatase Conjugated | 0.5mg |
| 32490 | Protein A/G, Peroxidase Conjugated | 0.5mg |

### Protein L, Recombinant

*Binds a wider range of Ig classes and subclasses, including all classes of IgG and single chain variable (ScFv) and Fab fragments.*

**Highlights:**

- Protein L is an immunoglobulin-binding protein that was originally derived from the bacteria *Peptostreptococcus magnus* but now is produced recombinantly in *E. coli*
- Has the unique ability to bind through kappa light chain interactions, including kappa I, III and IV in human and kappa I in mouse, without interfering with an antibody's antigen-binding site

**Ordering Information**

| Product # | Description | Pkg. Size |
|---|---|---|
| 21189 | Pierce Purified Recombinant Protein, Lyophilized | 1mg |
| 32420 | Protein L, Peroxidase Conjugated | 0.5mg |
| 29997 | Biotinylated Protein L | 0.5mg |

## Avidin-Biotin Products

The interaction between biotin (a vitamin) and avidin (hen egg white protein) has been exploited to produce a variety of applications. The noncovalent, high affinity of biotin for avidin ($K_a = 10^{15}\ M^{-1}$) has allowed us to create a line of products that can help you develop nonradioactive assay systems. With four biotin-binding sites per avidin molecule, this system allows more signal to be concentrated at the detection site. A similar assay scenario can be developed for DNA or RNA hybridization assays when a probe is biotinylated instead of an antibody. Below are just a few of the applications exploiting the avidin-biotin interaction even beyond assay development.

- ELISA
- Immunohistochemical staining
- Western blotting
- DNA hybridization assays
- Immunoprecipitation
- Affinity chromatography
- Fluorescent activated cell sorting (FACS)

**Comparison of Thermo Scientific NeutrAvidin Biotin-Binding Protein, Avidin and Streptavidin.**

| Protein | MW | pI | Carbohydrate |
|---|---|---|---|
| **Thermo Scientific NeutrAvidin Biotin-Binding Protein** | 60kDa | 6.3 | No |
| **Thermo Scientific Streptavidin** | 53kDa | 6.8-7.5 | No |
| **Thermo Scientific Avidin** | 67kDa | 10 | Yes |

### NeutrAvidin Products

*For ultralow nonspecific binding compared to avidin or streptavidin!*

Achieve better assay results with the low nonspecific binding properties of Thermo Scientific NeutrAvidin Protein. NeutrAvidin Biotin-Binding Protein is a deglycosylated form of avidin, so lectin binding is reduced to undetectable levels without losing biotin-binding affinity ($K_a = 10^{15}\ M^{-1}$).[1] NeutrAvidin Biotin-Binding Protein offers the advantage of a neutral pI to minimize nonspecific adsorption, along with lysine residues that remain available for derivatization or conjugation through amine-reactive chemistries. The molecular weight of NeutrAvidin Biotin-Binding Protein is approximately 60K. The specific activity for biotin-binding is approximately 14µg/mg of protein, which is near the theoretical maximum activity.

### Highlights:

- Near-neutral pI (6.3) and no glycosylation, unlike avidin
- No RYD recognition sequence like streptavidin
- Generally lower nonspecific binding than avidin and streptavidin
- Much lower price than streptavidin


**References**

1. Hiller, Y., *et al.* (1987). *Biochem. J.* **248**, 167-171.
Unson, M.D., *et al.* (1999). *J. Clin. Microbiol.* **37**, 2153-2157.
Wojciechowski, M., *et al.* (1999). *Clin. Chem.* **45**, 1690-1693.
Glover, B.P. and McHenry, C.S. (2001). *Cell* **105**, 925-934.
Guo, Y., *et al.* (2001). *J. Biol. Chem.* **276**, 45791-45799.
Claypool, S.M., *et al.* (2002). *J. Biol. Chem.* **27**, 28038-28050.


## Ordering Information

| Product # | Description | Features | Pkg. Size |
|---|---|---|---|
| **22831** | **NeutrAvidin, DyLight 405 Conjugated** | • Excellent photostability<br>• Intense emission provides superior sensitivity and requires less conjugate | 1mg |
| **22832** | **NeutrAvidin, DyLight 488 Conjugated** | | 1mg |
| **22842** | **NeutrAvidin, DyLight 594 Conjugated** | | 1mg |
| **22844** | **NeutrAvidin, DyLight 633 Conjugated** | | 1mg |
| **22848** | **NeutrAvidin, DyLight 680 Conjugated** | | 1mg |
| **22853** | **NeutrAvidin, DyLight 800 Conjugated** | | 1mg |
| **31000** | **NeutrAvidin Biotin-Binding Protein** | • pI that has been reduced to a neutral state | 10mg |
| **31050** | **NeutrAvidin Biotin-Binding Protein** | • Deglycosylated, so lectin binding is reduced to undetectable levels<br>• Can be used as a biotin blocking agent in tissues for histochemistry<br>• 11-17µg biotin bound/mg NeutrAvidin Protein | 100mg |
| **31001** | **NeutrAvidin Horseradish Peroxidase Conjugated** | • Better signal-to-noise ratio in assay systems<br>• 1-2 moles HRP/mole NeutrAvidin Protein<br>• 3-8µg biotin bound/mg conjugate | 2mg |
| **31002** | **NeutrAvidin Alkaline Phosphatase Conjugated** | • Lower nonspecific binding than streptavidin conjugates<br>• Better signal-to-noise ratio in assay systems<br>• 3-8µg biotin bound/mg conjugate | 2mg |
| **31006** | **NeutrAvidin Fluorescein Conjugated** | • Fluorescent-labeled NeutrAvidin Biotin-Binding Protein<br>• Absorption: 490nm; Emission 520nm<br>• ≥ 2 moles fluorescein/mole NeutrAvidin Protein | 5mg |
| **31007** | **EZ-Link Maleimide Activated NeutrAvidin Biotin-Binding Protein** | • Prepare NeutrAvidin conjugates of proteins/peptides<br>• Reacts spontaneously with free sulfhydryls in the pH range of 6.5-7.5<br>• 4-8 moles maleimide/mole NeutrAvidin Protein | 5mg |

## Streptavidin Products

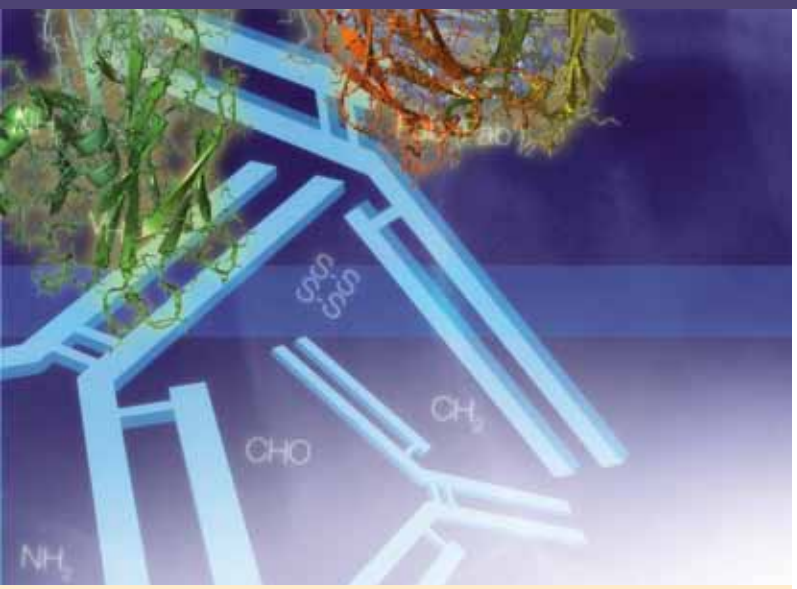
*Wide selection of conjugates for almost any biotin-based assay.*

Originally isolated from *Streptomyces avidinii*, streptavidin is a tetrameric biotin-binding protein that we produce and offer in recombinant form. Compared to the native protein, recombinant streptavidin is smaller that the native protein (MW 53K) and has a more neutral isoelectric point (pI 6.8-7.5). Thermo Scientific Streptavidin is carbohydrate-free and much less soluble in water than avidin, resulting in high binding affinity, capacity and specificity for biotinylated molecules. Streptavidin conjugates are useful for secondary detection in Western blotting, ELISA, and cell and tissue staining.

## Ordering Information

| Product # | Description | Features | Applications | Pkg. Size |
|---|---|---|---|---|
| 21122 | Streptavidin | • Lyophilized, stable powder<br>• No carbohydrate | • Immunoassay reagent when bound to biotinylated enzymes or when conjugated to enzymes | 1mg |
| 21125 | Streptavidin | • Much less soluble in water than avidin<br>• 13-22µg biotin bound/mg of protein | • Blocking protein for biotin-rich tissue sections (use at 0.1% for inhibition of endogenous biotin) | 5mg |
| 21135 | Streptavidin | • Recombinant | • Can be used with biotinylated enzymes (Product # 29339 or 29139) | 100mg |
| 21126 | Horseradish Peroxidase Conjugated | • 1-2 moles HRP/mole streptavidin | • Histochemistry | 1mg |
| 21124 | Horseradish Peroxidase Conjugated | • ≥ 100 peroxidase units/mg conjugate | • Western blotting | 2mg |
| 21127 | Horseradish Peroxidase Conjugated | • Lyophilized, stable powder<br>• 6-9µg biotin bound/mg conjugate | • Conti, L.R., *et al.* (2001). *J. Biol. Chem.* **276**, 41270-41278. | 5mg |
| 21324 | Alkaline Phosphatase Conjugated | • ≥ 3µg biotin bound/mg conjugate | • Histochemistry | 1mg |
| 21323 | Alkaline Phosphatase Conjugated | • ≥ 100 phosphatase units/mg conjugate | • Western blotting<br>• Harriman, G.R., *et al.* (1999). *J. Immunol.* **162**, 2521-2529.<br>• Nielsen, P.K., *et al.* (2000). *J. Biol. Chem.* **275**, 14517-14523. | 3mg |
| 21224 | Fluorescein (FITC) Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 490nm and 520nm<br>• 3-5 moles FITC/mole streptavidin | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 1mg |
| 21724 | Rhodamine (TRITC) Conjugated | • Fluorescently labeled streptavidin<br>• Excitation: 515-520nm and 550-555nm<br>• Emission: 575nm<br>• 1-3 moles TRITC/mole streptavidin | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 1mg |
| 21624 | Texas Red Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 595nm and 615nm | • Histochemical staining; can be used in double staining methods<br>• Fluorescence-activated cell sorting (FACS) | 1mg |
| 21627 | R-Phycoerythrin Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 480, 545 and 565nm and 578nm | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 1mL |
| 21629 | Allophycocyanin Conjugated | • Fluorescently labeled streptavidin<br>• Ex/Em: 650nm and 660nm | • Histochemical staining<br>• Fluorescence-activated cell sorting (FACS) | 0.5mL |
| 21120 | Hydrazide Activated | • Attaches streptavidin to oxidized carbohydrate residues on glycoproteins<br>• ≥ 4 moles hydrazide/mole streptavidin | • Used to create immunoassay reagents<br>• Localize glycoproteins on blot transfers, followed by detection with a biotinylated enzyme | 2mg |
| 21831 | Streptavidin, DyLight 405 Conjugated | • Ex/Em 400/420 | • Excellent photostability | • Fluorescence microscopy | 1mg |
| 21832 | Streptavidin, DyLight 488 Conjugated | • Ex/Em 493/518 | • Intense emission | • Flow cytometry | 1mg |
| 21842 | Streptavidin, DyLight 594 Conjugated | • Ex/Em 593/618 | sensitivity and requires | • ELISA | 1mg |
| 21844 | Streptavidin, DyLight 633 Conjugated | • Ex/Em 638/658 | less conjugate | • High content screening and other array platforms | 1mg |
| 21848 | Streptavidin, DyLight 680 Conjugated | • Ex/Em 692/712 | from pH 4-9 | | 1mg |
| 21851 | Streptavidin, DyLight 800 Conjugated | • Ex/Em 777/790 | | | 1mg |

# Antibody Labeling

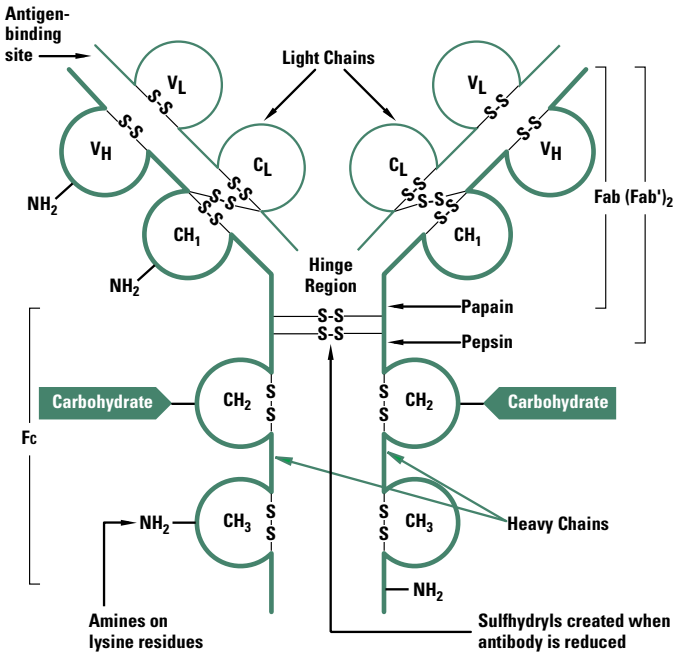



## Antibody Modification Sites

**Antibodies can be easily modified to contain labels such as biotin, fluorescent tags or enzymes to create reagents for Western blotting, ELISA, immunohistochemical staining and *in vivo* targeting. We offer tools for a variety of antibody modification strategies.**

Most antibody labeling methods involve one of four common target strategies. The most common target is primary amines ($-NH_2$), which are primarily on lysine residues. Primary amines are abundant, widely distributed and easily modified because of their reactivity and location on the antibody surface. The second most common target are sulfhydryls, which are generated by reducing disulfide bonds. These disulfide bonds join heavy and light polypeptide chains together in a manner that ensures proper antibody structure and antigen-binding function. Therefore, complete reduction of antibody disulfides by treatment with reducing agents will usually inactivate the antibody. However, with the proper conditions, it is possible to selectively reduce only the more labile disulfides between heavy chains in the hinge region of IgG molecules; the result is functional half-antibodies with sulfhydryls available for labeling. Such partial reduction of antibody disulfides usually results in sulfhydryl group labeling points that will not sterically hinder antigen binding.

Two other common targets are carboxyls, present on glutamic and aspartic acid residues, and carbohydrates. Carboxyls are abundant and easily accessible, but they do not react as readily as amines and coupling to them requires the crosslinker EDC. Carbohydrate moieties are present on the Fc portion of most polyclonal antibodies. This region usually can be labeled without altering antibody activity. Labeling carbohydrates is a two-step process because the carbohydrates must first be oxidized to create reactive aldehydes.




**Functional groups available on an antibody for labeling.**

Primary amines ($-NH_2$) are on lysine residues and the N-terminus. These are abundant and distributed over the entire antibody.

Sulfhydryl groups (–SH) are on cysteine residues and are generally involved in disulfide bonds. Free sulfhydryls can be formed by selectively reducing disulfide bonds in the hinge region of the antibody.

Carbohydrate residues containing *cis*-diols can be oxidized (–CHO) to create active aldehydes. These are localized to the Fc region on antibodies and are more abundant on polyclonal antibodies.

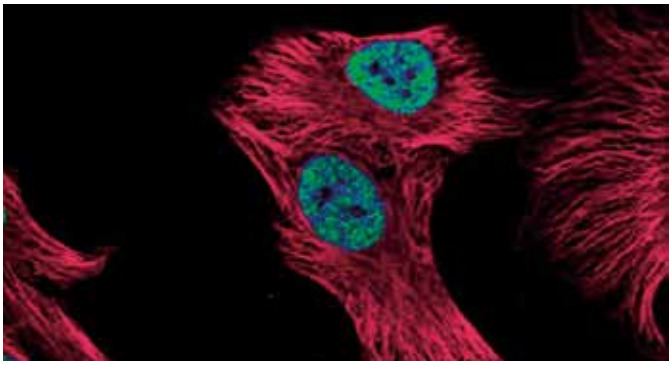
## DyLight Dyes

*Bright New Alternatives to Alexa Fluor®, CyDye™ and LI-COR Fluorescent Dyes.*

Thermo Scientific DyLight Dyes have absorption spectra ranging from 350nm to 770nm (Table 5) and match the principal output wavelengths of common fluorescence instrumentation. The DyLight Dyes exhibit higher fluorescence intensity and photostability than Alexa Fluor, CyDye and LI-COR Dyes in many applications and remain highly fluorescent over a broad pH range (pH 4-9). Additionally, the water solubility of the DyLight Dyes allows a high dye-to-protein ratio without precipitation during conjugation.

### Highlights:

- Available in both amine- and sulfhydryl-reactive chemistries for fast and efficient labeling of IgG or other proteins
- High water solubility
- Excellent photostability
- Compatible with common fluorescence instrumentation



**Thermo Scientific DyLight 488 and DyLight 633 Dyes exhibit outstanding fluorescence in structured illumination.** The uniform fluorescence intensity throughout the images demonstrates the outstanding brightness and photostability of DyLight 488 and 633 Dyes. Red: Alpha tubulin detected in HeLa cells with anti-tubulin monoclonal antibody and DyLight 633 Dye-conjugated secondary antibody (highly cross-adsorbed). Green: Histone H4 detected with anti-histone monoclonal antibody and DyLight 488 Dye-conjugated secondary antibody (highly cross-adsorbed). Blue: Nucleus counter-stained with fluorescent mounting media containing DAPI. Images were acquired with the Axio Imager Z1 and ApoTome™ Slider (Zeiss MicroImaging, Inc). The ApoTome Module provides confocal-like resolution allowing optical sectioning without using a pinhole (e.g., confocal). No image enhancement was performed.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **Amine-Reactive Dyes** | | |
| 46426 | DyLight 350 NHS Ester | 1mg |
| 46427 | DyLight 350 NHS Ester | 5 x 65µg |
| 46400 | DyLight 405 NHS Ester | 1mg |
| 46401 | DyLight 405 NHS Ester | 5 x 50µg |
| 46402 | DyLight 488 NHS Ester | 1mg |
| 46403 | DyLight 488 NHS Ester | 5 x 50µg |
| 46412 | DyLight 594 NHS Ester | 1mg |
| 46413 | DyLight 594 NHS Ester | 5 x 50µg |
| 46414 | DyLight 633 NHS Ester | 1mg |
| 46417 | DyLight 633 NHS Ester | 5 x 50µg |
| 46418 | DyLight 680 NHS Ester | 1mg |
| 46419 | DyLight 680 NHS Ester | 5 x 50µg |
| 46421 | DyLight 800 NHS Ester | 1mg |
| 46422 | DyLight 800 NHS Ester | 5 x 50µg |
| **Sulfhydryl-Reactive Dyes** | | |
| 46622 | DyLight 350 Maleimide | 1mg |
| 46600 | DyLight 405 Maleimide | 1mg |
| 46602 | DyLight 488 Maleimide | 1mg |
| 46608 | DyLight 594 Maleimide | 1mg |
| 46613 | DyLight 633 Maleimide | 1mg |
| 46618 | DyLight 680 Maleimide | 1mg |
| 46621 | DyLight 800 Maleimide | 1mg |

**Table 5. Spectral properties of Thermo Scientific DyLight Fluorescent Dyes.**

| Emission | DyLight Dye | Ex/Em* | ε† | Spectrally Similar Dyes |
|---|---|---|---|---|
| Blue | 350 | 353/432 | 15,000 | Alexa Fluor 350 Dye |
| Blue | 405 | 400/420 | 30,000 | Alexa Fluor 405 and Cascade Blue® Dyes |
| Green | 488 | 493/518 | 70,000 | Alexa Fluor 488, fluorescein and FITC Dyes |
| Red | 594 | 593/618 | 80,000 | Alexa Fluor 594 and Texas Red Dyes |
| Red | 633 | 638/658 | 170,000 | Alexa Fluor 633 Dye |
| Near-IR | 680 | 682/715 | 140,000 | Alexa Fluor 680 and Cy5.5 Dyes |
| Near-IR | 800 | 770/794 | 270,000 | IRDye® 800 Dye |

**Excitation and emission maxima in nanometers (± 4nm)*
*†Molar extinction coefficient ($M^{-1}$ $cm^{-1}$)*

## DyLight Antibody Labeling Kits

*Label and purify antibodies in one hour.*

The Thermo Scientific DyLight Antibody Labeling Kits were specifically developed for fast, efficient labeling of antibodies. Two convenient kit formats are available to accommodate varied labeling requirements. The Antibody Labeling Kits contain all necessary components to perform three separate labeling reactions using 1mg of IgG or similar quantities of other proteins. The Thermo Scientific DyLight Microscale Antibody Labeling Kits contain all the necessary components to perform five separate labeling reactions using 100µg of IgG.

The labeling kits use high-performance spin desalting columns to provide exceptional dye removal and antibody recovery (Figure 7).

**Highlights:**

- Fast – fluorescently label and purify protein in approximately one hour
- Amine-reactive dyes – label virtually any protein
- Pre-measured fluorescent dye – eliminate the time, waste and hassle associated with weighing dye
- Efficient non-reacted dye removal
- Minimal sample dilution
- Spin column format eliminates the need for column preparation, fraction screening and waiting for protein to emerge from column
- Easy protocol (Figure 8)

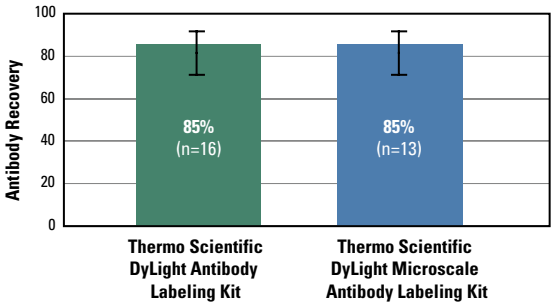



**Figure 7. Thermo Scientific DyLight Antibody Labeling Kits provide outstanding recovery.** The percent recovery for DyLight Antibody Labeling Kits is the average for 16 labeling reactions using three different antibodies. The percent recovery for DyLight Microscale Antibody Labeling Kits is the average for 13 labeling reactions using three different antibodies.

## Microscale Kits

*Contain sufficient reagents to label and purify 5 x 100µg of IgG.*

In addition to contents listed below, all Microscale Kits include:

- Reaction Buffer, 1mL
- Spin Columns, 5 each
- Microcentrifuge Collection Tubes, 10 each
- Purification Resin, 5mL

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 62276 | DyLight 350 Microscale Antibody Labeling Kit<br>DyLight 350 NHS Ester | Kit<br>5 vials |
| 53021 | DyLight 405 Microscale Antibody Labeling Kit<br>DyLight 405 NHS Ester | Kit<br>5 vials |
| 53025 | DyLight 488 Microscale Antibody Labeling Kit<br>DyLight 488 NHS Ester | Kit<br>5 vials |
| 53045 | DyLight 594 Microscale Antibody Labeling Kit<br>DyLight 594 NHS Ester | Kit<br>5 vials |
| 53047 | DyLight 633 Microscale Antibody Labeling Kit<br>DyLight 633 NHS Ester | Kit<br>5 vials |
| 53057 | DyLight 680 Microscale Antibody Labeling Kit<br>DyLight 680 NHS Ester | Kit<br>5 vials |
| 53063 | DyLight 800 Microscale Antibody Labeling Kit<br>DyLight 800 NHS Ester | Kit<br>5 vials |

## Antibody Labeling Kits

*Contain sufficient reagents to label and purify 3 x 1mg of IgG or similar quantities of other proteins.*

In addition to contents listed below, all Antibody Labeling Kits include:

- Reaction Buffer, 1mL
- Spin Columns, 6 each
- Microcentrifuge Collection Tubes, 12 each
- Purification Resin, 5mL

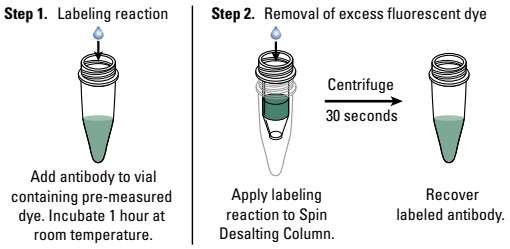



**Figure 8. Protocol summary for Thermo Scientific DyLight Antibody Labeling Kits.**

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 62275 | DyLight 350 Antibody Labeling Kit<br>DyLight 350 NHS Ester | Kit<br>3 vials |
| 53020 | DyLight 405 Antibody Labeling Kit<br>DyLight 405 NHS Ester | Kit<br>3 vials |
| 53024 | DyLight 488 Antibody Labeling Kit<br>DyLight 488 NHS Ester | Kit<br>3 vials |
| 53044 | DyLight 594 Antibody Labeling Kit<br>DyLight 594 NHS Ester | Kit<br>3 vials |
| 53046 | DyLight 633 Antibody Labeling Kit<br>DyLight 633 NHS Ester | Kit<br>3 vials |
| 53056 | DyLight 680 Antibody Labeling Kit<br>DyLight 680 NHS Ester | Kit<br>3 vials |
| 53062 | DyLight 800 Antibody Labeling Kit<br>DyLight 800 NHS Ester | Kit<br>3 vials |

## Fluorescein

*Amine-reactive derivatives of fluorescein dye.*

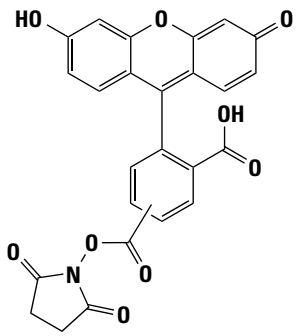
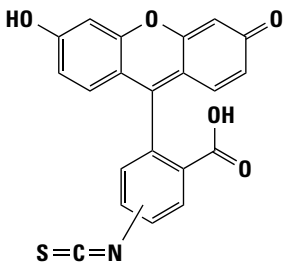
NHS-fluorescein and fluorescein isothiocyanate (FITC), two reactive derivatives of fluorescein dye, are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA. FITC is the base fluorescein molecule functionalized with an isothiocyanate reactive group (-N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive toward primary amine groups on proteins, peptides and other biomolecules. A succinimidyl-ester functional group attached to the fluorescein core, creating NHS-fluorescein, forms another common derivative that has much greater specificity toward primary amines in the presence of other nucleophiles and a more stable linkage following labeling.

Thermo Scientific Pierce Fluorescein is a mixture of isomers with reactive groups attached at the five and six positions of the bottom ring (See Structure). The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.

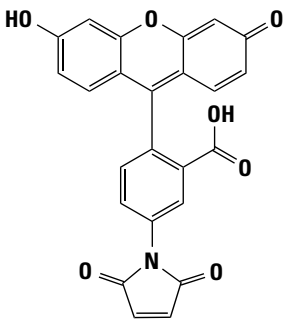
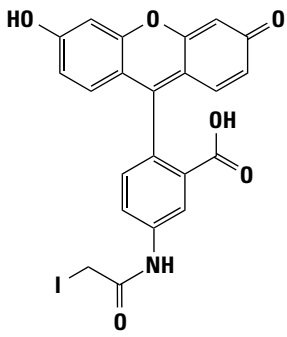
Fluorescein-5-maleimide and 5-Iodoacetamidofluorescein (5-IAF) are sulfhydryl-reactive derivatives of fluorescein dye. Fluorescein-5-maleimide is the base fluorescein molecule functionalized with a maleimide reactive group by replacing a hydrogen atom on the bottom ring of the structure. 5-IAF is the core fluorescein molecule functionalized with an iodoacetamide group. Both fluorescein derivatives are reactive toward sulfhydryl groups (e.g., reduced cysteine residues) on proteins, peptides and other biomolecules.

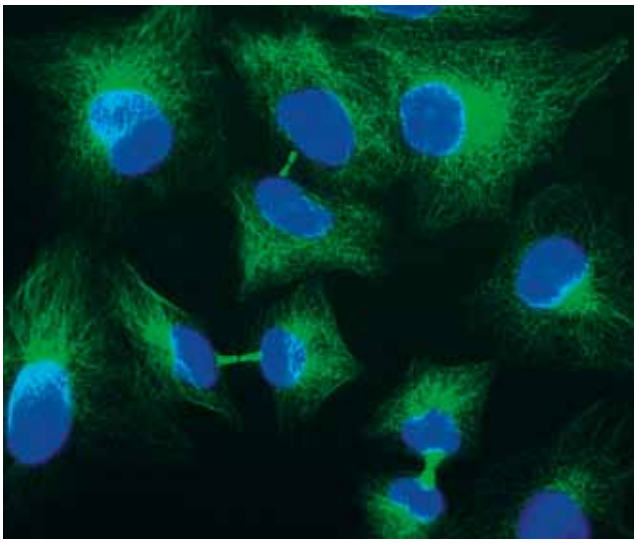
A derivative of fluorescein, DyLight 488 Fluor, has been tailored for various chemical and biological applications where greater photostability and fluorescence intensity, pH independence, and a narrower emission spectrum are required.

**Properties of Amine-reactive Fluorescein Dyes.**

| | NHS-Fluorescein | FITC |
|---|---|---|
| **Structure** |  **NHS-Fluorescein MW 473.39** |  **FITC MW 389.38** |
| **Alternative names** | 5/6-FAM SE | 5/6-FITC |
| **Chemical name** | 5/6-carboxyfluorescein succinimidyl ester | 5(6)-fluorescein isothiocyanate mixed isomer |
| **Molecular weight** | 473.4 | 389.2 |
| **Excitation source** | 488nm spectral line, argon-ion laser | 488nm spectral line, argon-ion laser |
| **Excitation wavelength** | 494nm | 494nm |
| **Emission wavelength** | 518nm | 518nm |
| **Extinction coefficient** | > 70,000/$M^{-1}$ $cm^{-1}$ | > 70,000/$M^{-1}$ $cm^{-1}$ |
| **CAS #** | 117548-22-8 | 27072-45-3 |
| **Purity** | > 90% by HPLC | > 95% by HPLC |
| **Solubility** | Soluble in DMF or DMSO | Soluble in aqueous buffers at pH > 6 |
| **Storage** | Desiccated at –20°C, protect from moisture, use only fresh solutions | Desiccated at –20°C, protect from moisture, use only fresh solutions |
| **Reactive groups** | NHS ester, reacts with primary amines at pH 7.0 to 9.0 | Isothiocyanate, reacts with primary amines at pH 7.0 to 9.0 |

**Properties of Sulfhydral-reactive Fluorescein Dyes.**

| | Fluorescein-5-maleimide | 5-Iodoacetamido-fluorescein |
|---|---|---|
| **Structure** |  <br>**Fluorescein-5-Maleimide**<br>**MW 427.36** |  <br>**5-IAF**<br>**5-Iodoacetamido-fluorescein**<br>**MW 515.25** |
| **Alternative names** | 5-MF, 5-maleimido-fluorescein | 5-IAF, 5-iodoacetamidofluorescein |
| **Chemical name** | 1H-Pyrrole-2,5-dione, 1-(3',6'-dihydroxy-3-oxospiro(isobenzofuran-1(3H),9'-(9H)xanthen-5-yl)- | Acetamide, N-(3',6'-dihydroxy-3oxospiro (isobenzofuran-1(3H), 9'-(9H)xanthen)-5-yl)-2-iodo |
| **Molecular weight** | 427.36 ±3 | 515.26 ±3 |
| **Excitation source** | 488nm spectral line, argon-ion laser | 488nm spectral line, argon-ion laser |
| **Excitation wavelength** | 494nm | 494nm |
| **Emission wavelength** | 518nm | 518nm |
| **Extinction coefficient** | ~ 68,000/$M^{-1}$ $cm^{-1}$ | > 80,000/$M^{-1}$ $cm^{-1}$ |
| **CAS #** | 75350-46-8 | 63368-54-7 |
| **Solubility** | Soluble in DMF or DMSO | Soluble in DMF; aqueous buffers at pH > 6 |
| **Storage** | Desiccated at –20°C, protect from moisture, use only fresh solutions | Desiccated at –20°C, protect from moisture, use only fresh solutions |
| **Reactive groups** | Maleimide, reacts with sulfhydryls at pH 6.5 to 7.5 | Iodoacetamide, reacts with sulfhydryls at pH 7.0 to 7.5 |



**The Thermo Scientific Pierce NHS-Fluorescein Antibody Labeling Kit (Product # 53029) produces ideal conjugates for immunofluorescence.** A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps® X-100 (Product # 28314). The cells were then probed with a 0.4µg/mL mouse anti-α-tubulin anti-body and 2µg/mL fluorescein-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse TS100 fluorescent microscope using Zeiss AxioCam™ camera and AxioVision™ software.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 46424 | FITC (Fluorescein Isothiocyanate) | 1g |
| 46425 | FITC (Fluorescein Isothiocyanate) | 100mg |
| 46409 | NHS-Fluorescein | 1g |
| 46410 | NHS-Fluorescein | 100mg |
| 53027 | FITC Antibody Labeling Kit<br>*Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.*<br>Includes: FITC<br>Borate Buffer<br>Spin Columns<br>Microcentrifuge Collection Tubes<br>Purification Resin | Kit<br><br><br>3 vials<br>1mL<br>6 each<br>12 each<br>5mL |
| 53029 | Fluorescein Antibody Labeling Kit<br>*Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.*<br>Includes: NHS Fluorescein<br>Borate Buffer<br>Spin Columns<br>Microcentrifuge Collection Tubes<br>Purification Resin | Kit<br><br><br>3 vials<br>1mL<br>6 each<br>12 each<br>5mL |
| 62245 | Fluorescein-5-Maleimide | 25mg |
| 62246 | 5-Iodoacetamido-fluorescein (5-IAF) | 25mg |

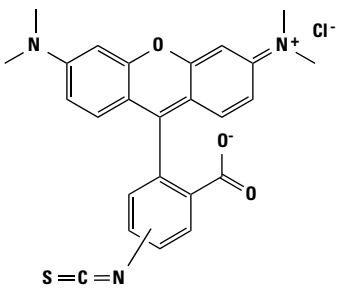
## Rhodamine

*Amine-reactive derivatives of rhodamine dye.*

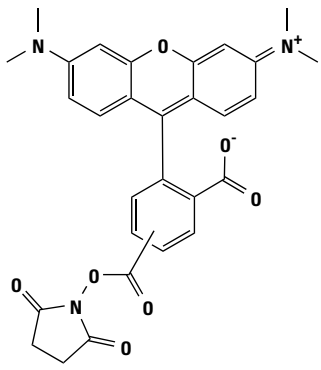
NHS-rhodamine and tetramethylrhodamine isothiocyanate (TRITC), two reactive derivatives of rhodamine dye, are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA.

TRITC is the base tetramethylrhodamine molecule functionalized with an isothiocyanate reactive group (-N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive toward amine and sulfhydryl groups on proteins, peptides and other biomolecules. A succinimidyl-ester functional group attached to the tetramethylrhodamine core, creating NHS-fluorescein, forms another common derivative that has much greater specificity toward primary amines in the presence of other nucleophiles and a more stable linkage following labeling. Texas Red Sulfonyl Chloride is a long-wavelength derivative of rhodamine that is modified with sulfonyl chloride for raction to primary amines.

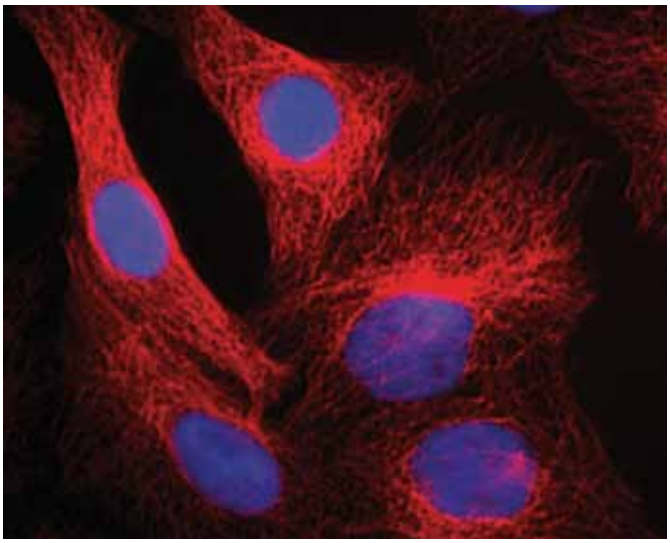
Thermo Scientific Pierce Rhodamine Dyes are a mixture of isomers with reactive groups attached at the five and six positions of the bottom ring (See Structure). The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.




**TRITC**
**MW 478.97**
**Em/Ex 544/572**




**NHS-Rhodamine**
**MW 527.52**
**Em/Ex 552/575**



**The Thermo Scientific NHS-Rhodamine Antibody Labeling Kit (Product # 53031) produces ideal conjugates for immunofluorescence.** A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps X-100 (Product # 28314). The cells were then probed with a 0.4µg/mL mouse anti-α-tubulin antibody and 2µg/mL rhodamine-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse TS100 fluorescent microscope using Zeiss AxioCam camera and AxioVison software.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **46112** | **TRITC (Tetramethylrhodamine Isothiocyanate)** | 10mg |
| **46406** | **NHS-Rhodamine** | 25mg |
| **53031** | **Rhodamine Antibody Labeling Kit**<br>*Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.*<br>Includes: NHS Rhodamine<br>Borate Buffer<br>Spin Columns<br>Microcentrifuge Collection Tubes<br>Purification Resin | Kit<br><br><br>3 vials<br>1mL<br>6 each<br>12 each<br>5mL |
| **46115** | **Texas Red Sulfonyl Chloride** | 10 x 1mg |

### Properties of Rhodamine-Derivative Dyes.

| | NHS-Rhodamine | TRITC | Texas Red Sulfonyl Chloride |
|---|---|---|---|
| **Alternative names** | 5/6-carboxy-tetramethyl-rhodamine succinimidyl ester, 5/6-TAMRA SE | 5(6)-tetramethyl-rhodamine isothiocyanate mixed isomer, 5/6-TRITC, TMR | Sulforhodamine 101 sulfonyl chloride, Texas Red-X, T1905, T353 |
| **Molecular weight** | 527.5 | 479.0 (chloride salt); 443.5 (no Cl) | 625.15 |
| **Excitation source** | 546nm spectral line, mercury-arc lamp; 543nm spectral line, He-Ne laser | 546nm spectral line, mercury-arc lamp; 543nm spectral line, He-Ne laser | 568nm spectral line, Ar-Kr laser; 594nm spectral line orange He-Ne laser |
| **Excitation wavelength** | 552nm | 544nm | 596nm |
| **Emission wavelength** | 575nm | 572nm | 615nm |
| **Extinction coefficient** | >80,000/M cm | >80,000/M cm | ~80,000/M cm |
| **CAS #** | 246256-50-8 | 95197-95-8 | 82354-19-6 |
| **Purity** | > 90% by HPLC | > 85% by HPLC | > 85% by HPLC |
| **Solubility** | Soluble in DMF or DMSO | Soluble in DMF or DMSO | Soluble in DMF or methylene chloride (not stable in DMSO) |
| **Storage** | Desiccated at –20°C, protect from moisture, use only fresh solutions | Desiccated at –20°C, protect from moisture, use only fresh solutions | Desiccated at –20°C, protect from moisture, use only fresh solutions |
| **Reactive groups** | NHS ester, reacts with primary amines at pH 7.0 to 9.0 | Isothiocyanate, reacts with primary amines at pH 7.0 to 9.0 | Sulfonyl chloride, reacts with primary amines at pH 7.0 to 9.0 |

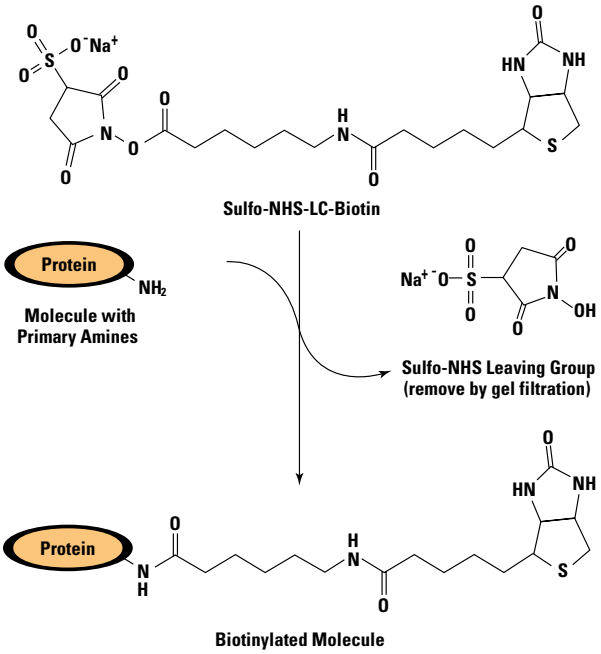
## EZ-Link Biotinylation Kits

*Everything you need to rapidly and successfully biotinylate purified proteins.*

Choose one of these easy-to-use kits and be confident that you have all the tools you need to efficiently biotinylate an antibody or other protein. The kits provide sufficient biotinylation reagent for eight to 10 labeling reactions of 1-10mg of protein each. Each kit also includes an appropriate labeling reaction buffer and 10 Thermo Scientific Zeba Desalt Spin Columns for efficient clean-up of the labeled protein. Finally, the HABA dye and avidin kit components allow one to determine the extent of labeling (i.e., the biotin:protein molar ratio).

### Highlights:

- NHS-ester biotins react specifically with primary amines (N-terminus and side chain of lysine residues), resulting in stable amide bond
- Sufficient reagents for 10 labeling reactions (8 reactions for Product # 21455)
- Labels 1-10mg protein in 0.5-2mL per reaction
- Sulfonate form of NHS ester (or the PEG spacer arm for Product # 21455) enhances water solubility of the biotin reagent
- Zeba Desalt Spin Columns (5mL) provide rapid sample clean up and excellent protein recovery (faster than dialysis and gravity-flow gel filtration, and much better protein recovery and desalting efficiency than diafiltration)
- Includes reagents and protocol for determining labeling efficiency




**Sulfo-NHS-LC-Biotin reaction scheme.**

### Sulfo-NHS-Biotin (Product # 21425)

- Shortest spacer arm and simplest biotin reagent
- Adds smallest possible mass to labeled protein

### Sulfo-NHS-LC-Biotin (Product # 21435)

- Historically the most popular and widely applied biotin reagent
- Extended spacer arm (compared to Sulfo-NHS-Biotin) minimizes possibility of steric hindrance for avidin or streptavidin binding
- Useful for other applications (e.g., cell-surface labeling) besides labeling purified proteins

### Sulfo-NHS-SS-Biotin (Product # 21445)

- Cleavable disulfide bond in spacer arm (can be reduced with DTT or other reducing agent to release the labeled protein avidin or streptavidin in protein interaction or affinity applications)

### NHS-PEG$_4$-Biotin (Product # 21455)

- Polyethylene glycol (PEG) spacer arm enhances water solubility
- Hydrophilic PEG spacer confers solubility to labeled protein (i.e., antibodies labeled with this reagent are less likely to aggregate and precipitate during long-term storage than those labeled with other biotin reagents)

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 21425 | EZ-Link Sulfo-NHS-Biotinylation Kit<br>Includes: EZ-Link Sulfo-NHS-Biotin<br>BupH PBS (pack makes 500mL)<br>Zeba Desalt Spin Columns, 5mL<br>HABA (10mM)<br>Avidin, Affinity-purified | Kit<br>25mg<br>1 pack<br>10 each<br>1mL<br>10mg |
| 21435 | EZ-Link Sulfo-NHS-LC-Biotinylation Kit<br>Includes: EZ-Link Sulfo-NHS-Biotin<br>Non-reagent contents same as Product # 21425 | Kit<br>25mg |
| 21445 | EZ-Link Sulfo-NHS-SS-Biotinylation Kit<br>Includes: EZ-Link Sulfo-NHS-SS-Biotin<br>Non-reagent contents same as Product # 21425 | Kit<br>25mg |
| 21455 | EZ-Link NHS-PEG$_4$-Biotinylation Kit<br>Includes: No-Weigh NHS-PEG$_4$-Biotin<br>Non-reagent contents same as Product # 21425 | Kit<br>8 x 2mg |

## EZ-Link Micro Biotinylation Kits

*Just what you need to biotinylate 50-200µg antibody or other protein.*

Finally! Biotinylation kits that provide reagents and accessories suited for labeling small amounts of protein. The Thermo Scientific EZ-Link Micro Biotinylation Kits are similar to the kits described on page 48, the reagent in these kits is supplied in a convenient No-Weigh Microtube format and the kits include smaller Thermo Scientific Zeba Desalt Spin Columns that are ideal for small-scale labeling of commercial antibodies and other samples that are available in limited amounts.

**Highlights:**

- NHS-ester biotins react specifically with primary amines (N-terminus and side chain of lysine residues), resulting in stable amide bond
- Sufficient reagents for eight labeling reactions
- Labels 50-200µg protein in 200-700µL per reaction
- Sulfonate form of NHS ester (or the PEG spacer arm for Product # 21955) enhances water solubility of the biotin reagent
- Zeba Desalt Spin Columns (2mL) provide rapid sample clean up and excellent protein recovery (faster than dialysis and gravity-flow gel filtration, and much better protein recovery and desalting efficiency than diafiltration)
- Micro Biotinylation Kits do NOT include reagents and protocol for determining labeling efficiency because it would use up most of the small amount of labeled sample

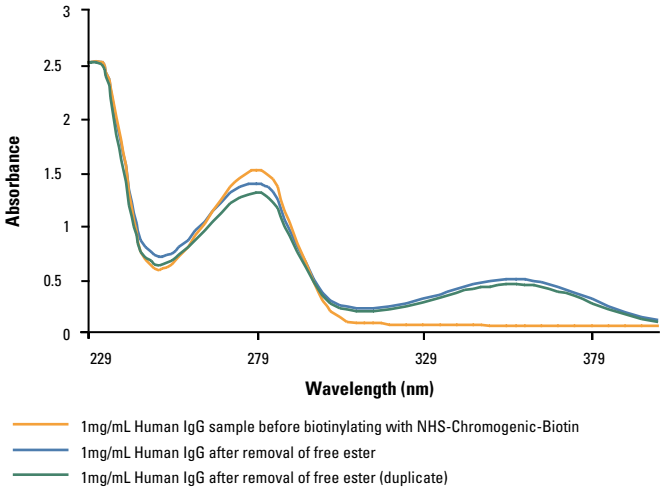
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 21925 | EZ-Link Micro Sulfo-NHS-Biotinylation Kit<br>Includes: No-Weigh Sulfo-NHS-Biotin<br>BupH PBS (pack makes 500mL)<br>Zeba Desalt Spin Columns, 2mL | Kit<br>8 x 2mg<br>1 pack<br>10 each |
| 21935 | EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit<br>Includes: No-Weigh Sulfo-NHS-LC-Biotin<br>Non-reagent contents same as Product # 21925 | Kit<br>8 x 1mg |
| 21945 | EZ-Link Micro Sulfo-NHS-SS-Biotinylation Kit<br>Includes: No-Weigh Sulfo-NHS-SS-Biotin<br>Non-reagent contents same as Product # 21925 | Kit<br>8 x 1mg |
| 21955 | EZ-Link Micro NHS-$PEG_4$-Biotinylation Kit<br>Includes: No-Weigh NHS-$PEG_4$-Biotin<br>Non-reagent contents same as Product # 21925 | Kit<br>8 x 2mg |

## EZ-Link NHS-Chromogenic-Biotinylation Kit

*A protein labeling kit with a unique biotinylation reagent.*

This convenient kit includes all components needed to label five purified protein samples (1-10mg each) with NHS-Chromogenic-Biotin, a distinctive reagent with a long spacer arm and built-in chromophore for measuring labeling efficiency. Antibodies and other proteins are easily labeled where primary amines occur on their surface. After sample cleanup with a Zeba Desalt Spin Column that is included in the kit, the sample absorbance at 354nm can be used to directly calculate the extent of biotinylation.




**Labeling of human IgG with Thermo Scientific EZ-Link NHS-Chromogenic-Biotin.** A 1mg/mL human IgG sample in 1mL total volume of phosphate-buffered saline was modified with EZ-Link NHS-Chromogenic-Biotin. Samples were prepared in duplicate and desalted using 5mL Thermo Scientific Zeba Desalt Spin Columns. Note the absorption at 354nm from the covalent addition of the chromogenic biotinylation agent and the minimal loss of protein as illustrated by the 280nm absorption maxima.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 21625 | EZ-Link NHS-Chromogenic-Biotinylation Kit<br>Includes :NHS-Chromogenic-Biotin<br>BupH PBS (pack makes 500mL)<br>Zeba Desalt Spin Columns, 5mL<br>DMF (N,N-dimethylformamide) | Kit<br>10mg<br>1 pack<br>5 each<br>4mL |

## EZ-Link Solid-Phase Biotinylation Kits

*An easier way to biotinylate IgG antibodies.*

This innovative antibody-labeling system uses nickel-chelated agarose to temporarily immobilize antibody molecules via their histidine-rich Fc regions. Once held in place on the gel, the antibody can be biotinylated at either sulfhydryl groups (after mild reduction or disulfide bonds) or primary amines. Excess labeling reagent and byproducts are then washed away before recovering the labeled and purified antibody from the gel using a mild imidazole solution. No gel filtration or dialysis are needed. Four kits are available for small (0.1-1mg) or large (1-10mg) antibody samples using either amine-directed (NHS ester) or sulfhydryl-directed (maleimide) labeling reagents.

These kits contain our unique No-Weigh Single-Dose Microtube Packaging. A single sealed microtube containing 2mg of reagent is reconstituted for each biotinylation. The exclusive No-Weigh Packaging allows access to fresh reagent on-demand for each solid-phase biotinylation reaction.

### Highlights:

- **Fast labeling and purification** – the entire procedure takes only one hour (two hours for sulfhydryl labeling kits)
- **Easy removal of spent and excess labeling reagent** – simply wash away the reaction byproducts – no need for dialysis or gel filtration
- **No dilution effects** – solid-phase method allows initially dilute antibodies to be recovered in a smaller volume after labeling
- **Optimized protocols** – specific protocols for antibody ensure appropriate level of labeling (2-5 biotins per antibody molecule), minimizing possibility of inactivation caused by overlabeling
- **Sufficient reagents for eight biotin-labeling experiments** – No-Weigh Single-Dose Microtube Packaging ensures that the biotin reagent is fully active for eight separate experiments

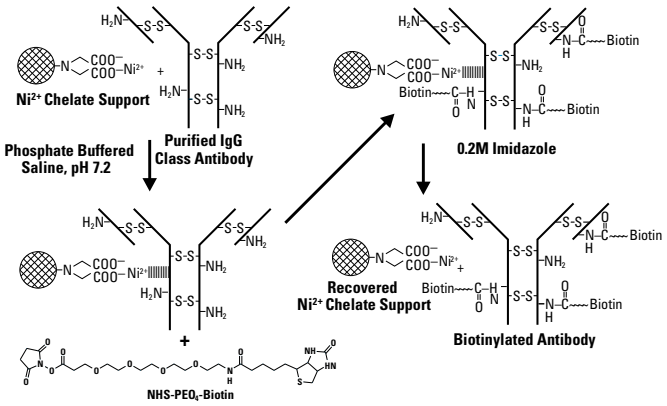
**Convenient kit sizes and labeling chemistries available.**

| Antibody Sample Size | Amine-directed Labeling ($NHS\text{-}PEG_4$-Biotin) | Sulfhydryl-directed Labeling (Maleimide-$PEG_4$-Biotin) |
|---|---|---|
| 0.1-1mg IgG | Product # 21450 | Product # 21930 |
| 1-10mg IgG | Product # 21440 | Product # 21920 |

**Step 1. Immobilize the IgG**
- a. 1mL Ni-IDA column (for 1-10mg of IgG)
- b. SwellGel® Nickel Chelated Disc (for 0.1-1mg IgG)†

**Step 2. Add the labeling reagent(s) to the immobilized IgG**
- a. $NHS\text{-}PEG_4$-Biotin for amine-directed reactions
- b. TCEP, followed by Maleimide-$PEG_2$-Biotin for sulfhydryl-directed reactions

**Step 3. Elute the biotinylated IgG with 0.2 M imidazole**




**Solid-phase protocol summary for labeling amines.**

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **21440** | **EZ-Link NHS-PEG Solid-Phase Biotinylation Kit – Pre-Packed Column** | Kit |
| | *Biotinylates antibodies and other proteins that bind to the nickel-chelated support provided. A 1mL column biotinylates 1-10mg of antibody and can be re-used 10 times.* | |
| | Includes: Immobilized Nickel Chelated Column | 1mL, pre-packed |
| | BupH Phosphate Buffered Saline (makes 500mL) | 1 pack |
| | No-Weigh $NHS\text{-}PEG_4$-Biotin | 8 x 2mg |
| | 4 M Imidazole Stock Solution | 5mL |
| **21450** | **EZ-Link NHS-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns** | Kit |
| | *Biotinylate antibodies and other proteins that bind to the rehydrated nickel-chelated discs provided. Each disc can biotinylate 100-1000µg of antibody.* | |
| | Includes: SwellGel Nickel Chelated Discs | 10 pack |
| | Mini-Spin Columns | 10 pack |
| | Microcentrifuge Tubes (2mL) | 30 pack |
| | BupH Phosphate Buffered Saline (makes 500mL) | 1 pack |
| | No-Weigh $NHS\text{-}PEG_4$-Biotin | 8 x 2mg |
| | 4 M Imidazole Stock Solution | 5mL |
| **21920** | **EZ-Link Maleimide-PEG Solid-Phase Biotinylation Kit – Pre-Packed Column** | Kit |
| | *Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. A 1mL column biotinylates 1-10mg of antibody and can be re-used 10 times.* | |
| | Includes: Bond-Breaker® TCEP Solution, Neutral pH | 5mL |
| | Immobilized Nickel Chelated Column | 1mL, pre-packed |
| | BupH Tris Buffered Saline (makes 500mL) | 1 pack |
| | No-Weigh Maleimide-$PEG_2$-Biotin | 8 x 2mg |
| | 4 M Imidazole Stock Solution | 5mL |
| **21930** | **EZ-Link Maleimide-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns** | Kit |
| | *Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. Each disc can biotinylate 100-1,000µg of antibody.* | |
| | Includes: SwellGel Nickel Chelated Discs | 10 pack |
| | Bond-Breaker TCEP Solution, Neutral pH | 5mL |
| | Mini-Spin Columns | 10 pack |
| | Microcentrifuge Tubes (2mL) | 30 pack |
| | BupH Tris Buffered Saline (makes 500mL) | 1 pack |
| | No-Weigh Maleimide-$PEG_2$-Biotin | 8 x 2mg |
| | 4 M Imidazole Stock Solution | 5mL |

## Enzyme Labeling

### Maleimide Activation

The heterobifunctional cross-linker SMCC (Product # 22360) and its water-soluble analog Sulfo-SMCC (Product # 22322) have good general utility in preparing immunologically active horseradish peroxidase or alkaline phosphatase conjugates. They are most useful when preparing conjugates of reduced IgG and $F(ab')_2$, because these methods involve the initial step of preparing a maleimide-activated (sulfhydryl-reactive) enzyme derivative. Studies have shown that the two-step maleimide method is superior to glutaraldehyde or *meta*-periodate methods for enzyme conjugation (Figure 8). The maleimide method gives higher yields with less polymerization, producing a conjugate preparation with superior immunoassay characteristics.

Maleimide-activated enzymes can be prepared using the heterobifunctional cross-linker Sulfo-SMCC. This reagent contains an *N*-hydroxy-sulfosuccinimide (Sulfo-NHS) functional group and a maleimide functional group and it is water-soluble due to the presence of the sulfonate ($–SO_3–$) group on the *N*-hydroxysuccinimide ring. The sulfonate group also contributes to the stability of the molecule in aqueous solution. A study of the hydrolysis rate of the maleimide functional group from Sulfo-SMCC showed that it is less prone to hydrolysis to the maleamic acid than the non-sulfonated SMCC. The maleimide groups of Sulfo-SMCC exhibit no decomposition at pH 7 at 30˚C within 6 hours. The Sulfo-NHS ester group reacts with primary amines on the enzyme surface to form a stable amide bond. After this first step of conjugation, the enzyme will have maleimide groups on its surface that react optimally toward sulfhydryl groups between pH 6.5 and 7.5 to form stable thioether bonds. Maleimide-mediated conjugation strategies are summarized in Figure 9.

Two reagents, Mercaptoethylamine•HCl (Product # 20408) and SATA (Product # 26102), are available to produce free sulfhydryls on macromolecules for conjugation to the maleimide-activated enzymes. For labeling antibody molecules, mild reduction with Mercaptoethylamine•HCl (MEA) results in two half-antibody fragments containing free sulfhydryl groups in the hinge region. Labeling in this area is advantageous because it directs the modification away from the antigen-binding region. Native proteins lacking a free sulfhydryl on their surface can be reacted with SATA to generate blocked sulfhydryl groups. The SATA molecule reacts with primary amines via its NHS ester end to form stable amide linkages. The acetylated sulfhydryl group (blocked) is stable until treated with hydroxylamine to generate the free sulfhydryls.

We offer a stable, preactivated enzyme derivative that is reactive toward sulfhydryl (–SH) groups, EZ-Link Maleimide Activated and Horseradish Peroxidase (Product # 31485). These products eliminate the first step of the two-step maleimide method, simplifying and facilitating the conjugation protocol, while saving several hours. They can be used to prepare enzyme conjugates directly from proteins, peptides or other ligands that contain a free –SH group. Two reagents, SATA and mercaptoethylamine•HCl, are also included in the kit formats to produce free sulfhydryls on macromolecules for conjugation.

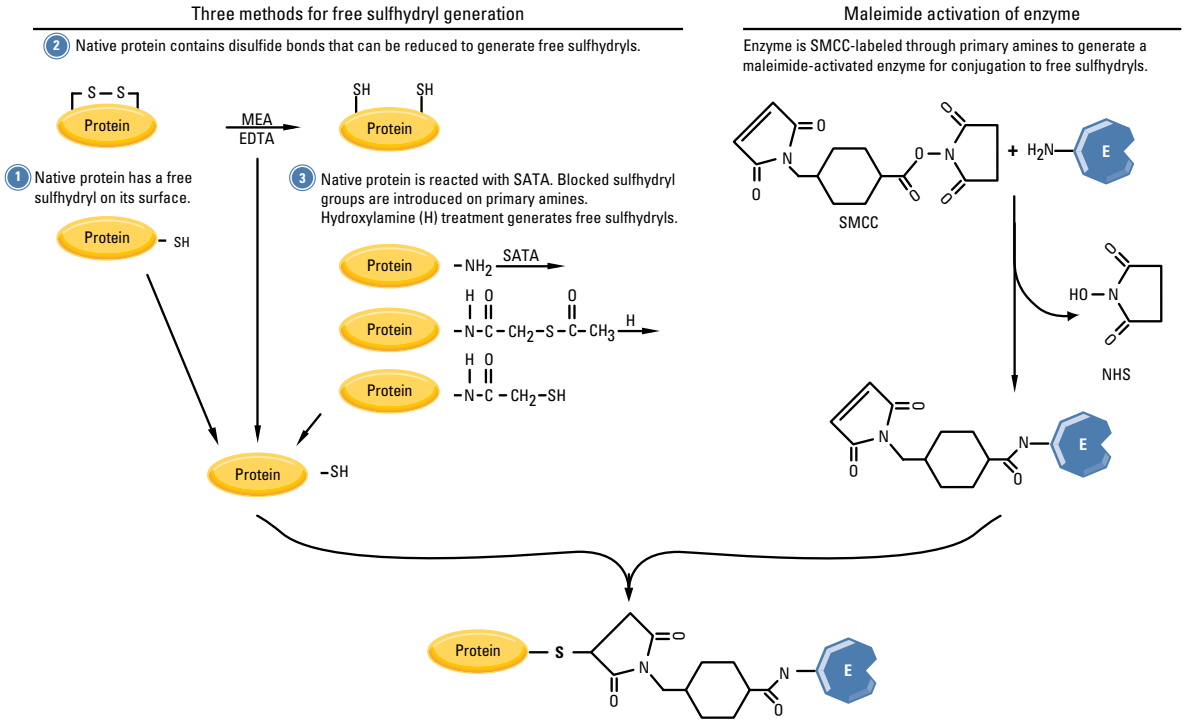



**Figure 9. Three strategies for maleimide-mediated conjugation of enzymes.**

## Horseradish Peroxidase (HRP)

*Its higher specific enzyme activity makes it the enzyme of choice.*

### Highlights:

- Superior to alkaline phosphatase and β-galactosidase conjugates because of its higher specific enzyme activity
- Small size (40kDa) allows excellent cellular penetration
- Variety of substrates available
- Ideal in blotting and cytochemistry applications
- Used as the reporter enzyme for SuperSignal Chemiluminescent Substrates

**References**

Cordell, J.L., *et al.* (1984). *J. Histochem. Cytochem.* **32**, 219-229.
Hosoda, H., *et al.* (1987). *Chem. Pharm. Bull.* **35**, 3336-3342.
Passey, R.B., *et al.* (1977). *Clin. Chem.* **23(1)**, 131-139.
Porstmann, B., *et al.* (1985). *J. Immunol. Methods.* **79**, 27-37.
Samoszuk, M.K., *et al.* (1989). *Antibody, Immunoconjugates and Radiopharmaceuticals* **2**, 37-46.
Wordinger, R.J., *et al.* (1987). Manual of Immunoperoxidase Techniques, 2nd Edition. Chicago: American Society of Clinical Pathologists Press, pp. 23-24.
Yolken, R.H. (1982). *Rev. Infect. Dis.* **4(1)**, 35-68.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **31490** | Horseradish Peroxidase | 10mg |
| **31491** | Horseradish Peroxidase | 100mg |

## Alkaline Phosphatase (AP)

*A highly sensitive enzyme for ELISA and immunohistochemical applications.*

### Highlights:

- Purified form – ready to conjugate without prior dialysis
- Activity is not affected by exposure to antibacterial agents, such as sodium azide or thimerosal
- Specific activity > 2000 units/mg
- One unit is defined as the amount that will hydrolyze 1.0µmol of *p*-nitrophenyl phosphate per minute at 37°C in 1.0 M diethanolamine, 0.5mM $MgCl_2$, pH 7.8

**Specific activity per mg protein.**

| Buffer | 25°C | 37°C |
|---|---|---|
| 0.1M Glycine, 1.0mM $ZnCl_2$, 1.0mM $MgCl_2$, 6.0mM *p*-Nitrophenyl phosphate, pH 10.4 | > 500 | > 1000 |
| 1.0M Diethanolamine, 0.5mM $MgCl_2$, 15mM *p*-Nitrophenyl phosphate, pH 9.8 | > 1000 | > 2000 |

**References**

Bulman, A.S. and Heyderman, E. (1981). *J. Clin. Pathol.* **34**, 1349-1351.
Cordell, J.L., *et al.* (1984). *J. Histochem. Cytochem.* **32**, 219-229.
Yolken, R.H. (1982). *Rev. Infect. Dis.* **4**, 35-68.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **31391** | Alkaline Phosphatase<br>Calf intestinal. Supplied in Tris Buffer, pH ~7 Triethanolamine, 1mM $MgCl_2$, 3 M NaCl, pH 7.6 | 20mg |
| **31392** | Alkaline Phosphatase | 100mg |

## EZ-Link HRP Enzyme Labeling Kits and Reagents

Thermo Scientific EZ-Link Pre-Activated HRP makes it easy to convert almost any protein to a detection reagent. Simple protocols with each EZ-Link Kit or Activated Enzyme turn a chemical chore into a painless process.

Horseradish peroxidase (HRP) is the most common enzyme used for immunoassay detection systems. This enzyme catalyzes reactions with substrates to form soluble color responses or colored precipitates, or to generate the chemical emission of light (chemiluminescence). Enzyme conjugates make stable assay reagents and can be stored for long periods at -20°C.

In one hour at room temperature, you can conjugate:

- HRP to a primary amine group (–$NH_2$) with the EZ-Link Plus Activated Peroxidase Kit (see page 55)
- HRP to a free sulfhydryl (–SH) group with the EZ-Link Maleimide Activated HRP Kit

## EZ-Link Maleimide Horseradish Peroxidase

*Make quick and easy enzyme conjugates.*

The Thermo Scientific EZ-Link Maleimide Activated HRP Kit can be used to directly prepare antibody conjugates via sulfhydryl groups. This kit eliminates the first step of the two-step maleimide method, making conjugate preparation much simpler. The kit is supplied with a stable, preactivated HRP.

The HRP conjugates can be purified by gel filtration chromatography, ultrafiltration or dialysis, depending upon the size of the conjugated protein. Alternatively, use Thermo Scientific Pierce Conjugate Purification Kit (Product # 44920) to separate unreacted enzyme from the conjugate.

### Highlights:

- Prepare HRP conjugates from proteins that contain a free sulfhydryl
- Includes 2-mercaptoethylamine to generate free sulfhydryls from disulfide bonds
- Includes SATA to add free sulfhydryls to lysine residues

**References**


Choi, J.Y., *et al.* (2002). *J. Biol. Chem.* **277**, 21630-21638.
Seo, Y.R., *et al.* (2002). *Proc. Natl. Acad. Sci. USA* **99**, 14548-14553.
Yoo, J.H., *et al.* (2004). *J. Biol. Chem.* **279**, 848-858.


### Ordering Information

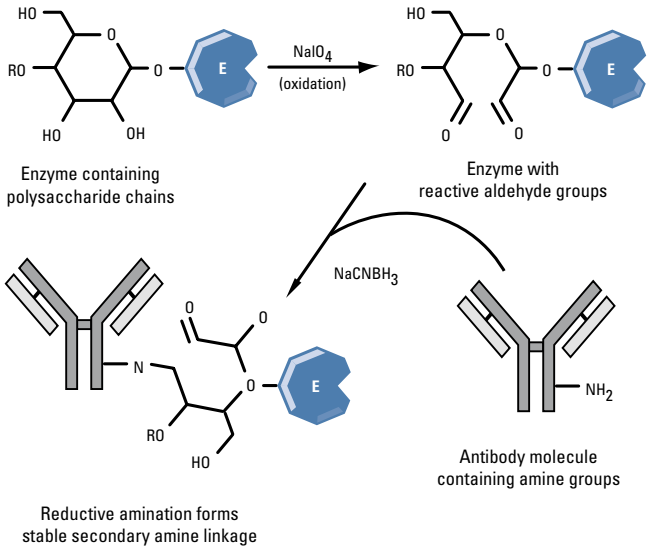
| Product # | Description | Pkg. Size |
|---|---|---|
| **31485** | **EZ-Link Maleimide Activated Horseradish Peroxidase** | 5mg |
| **31494** | **EZ-Link Maleimide Activated Horseradish Peroxidase Kit** | Kit |
| | Includes: EZ-Link Maleimide Activated Horseradish Peroxidase | 5mg |
| | Activated Horseradish Peroxidase Conjugation Buffer | 20mL |
| | 2-Mercaptoethylamine•HCl | 6mg |
| | SATA | 2mg |
| | Dimethylformamide | 1mL |
| | Hydroxylamine•HCl | 5mg |
| | Polyacrylamide Desalting Column | 1 x 10mL |
| **23460** | **Protein-Coupling Handle Addition Kit** | Kit |
| | Includes: SATA | 2mg |
| | Hydroxylamine•HCl | 5mg |
| | Conjugation Buffer Stock (10X) | 20mL |
| | BupH Pack PBS | 1 pack |
| | Dimethylformamide (DMF) | 1mL |
| | Dextran Desalting Column | 1 x 5mL |
| | Column Extender | 1 |
| | Ellman's Reagent (DTNB) | 2mg |
| | Cysteine•HCl $H_2O$ | 20mg |

### Periodate

Glycoproteins such as horseradish peroxidase and glucose oxidase and most antibody molecules can be activated for conjugation by treatment with periodate. Oxidizing polysaccharide residues in a glycoprotein with sodium periodate provides a mild and efficient way of generating reactive aldehyde groups for subsequent conjugation with amine- or hydrazide-containing molecules via reductive amination. Some selectivity of monosaccharide oxidation may be accomplished by regulating the concentration of periodate in the reaction medium. In the presence of 1mM sodium periodate, sialic acid groups are specifically oxidized at adjacent hydroxyl residues, cleaving off two molecules of formaldehyde and leaving one aldehyde group. At higher concentrations of sodium periodate (10mM or greater), other sugar residues will be oxidized at points where adjacent carbon atoms contain hydroxyl groups. This reaction should be performed in the dark to prevent periodate breakdown and for a limited period of time (15-30 minutes) to avoid loss of enzymatic activity.

Crosslinking with an amine-containing protein takes place under alkaline pH conditions through the formation of Schiff base intermediates. These relatively labile intermediates can be stabilized by reduction to a secondary amine linkage with sodium cyanoborohydride. Reductive amination has been done using sodium borohydride or sodium cyanoborohydride; however, cyanoborohydride is the better choice because it is more specific for reducing Schiff bases and will not reduce aldehydes. Small blocking agents such as lysine, glycine, ethanolamine or Tris can be added after conjugation to quench any unreacted aldehyde sites. Ethanolamine and Tris are the best choices for blocking agents because they contain hydrophilic hydroxyl groups with no charged functional groups.

The pH of the reductive amination reaction can be controlled to affect the efficiency of the cross-linking process and the size of the resultant antibody-enzyme complexes formed. At physiological pH, the initial Schiff base formation is less efficient and conjugates of lower molecular weight result. At more alkaline pH (i.e., pH 9-10), Schiff base formation occurs rapidly and with high efficiency, resulting in conjugates of higher molecular weight and greater incorporation of enzyme when oxidized enzyme is reacted in excess. Low molecular weight conjugates may be more optimal for immunohistochemical staining or blotting techniques in which penetration of the complex through membrane barriers is an important consideration. Washing steps also more effectively remove excess reagent if the conjugate is of low molecular weight, thus maintaining low background in an assay. By contrast, conjugates of high molecular weight are more appropriate for ELISA procedures in a microplate format, where high sensitivity is important and washing off excess conjugate is not a problem.




**Conjugation scheme for periodate oxidation and subsequent reductive amination.**

## EZ-Link Plus Activated Peroxidase Kit

*Amine-reactive HRP with the highest conjugation yields.*

Thermo Scientific EZ-Link Plus Activated Peroxidase is an amine-reactive HRP derivative that provides coupling efficiencies of greater than 95%. Other amine-reactive chemistries, like glutaraldehyde, tend to polymerize and produce lower amounts of viable conjugate. Sugar residues present on HRP have been oxidized with periodate to produce aldehydes that react with primary amines.

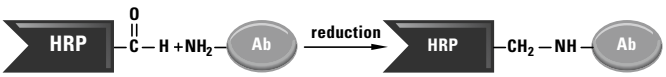
Our EZ-Link Plus Activated Peroxidase is mixed with the protein to be coupled at a pH compatible with your protein or antibody. After incubating the reaction for one to two hours, the conjugate linkage is reduced and the activated HRP quenched with ethanolamine. The conjugate is purified by desalting or by dialysis. If you are preparing an antibody-HRP conjugate, use the Thermo Scientific Pierce Conjugate Purification Kit (Product # 44920) to separate unreacted enzyme from the conjugate.

### Highlights:

- Reacts with readily accessible primary amines to form a covalent secondary amine bond
- Can be stored for at least 12 months at –20°C
- Consistent conjugation yields reliable conjugates
- Enzyme activity is 120-200 units/mg

**Suggested antibody-HRP dilutions for colorimetric substrates.**

| Technique | Working Range |
|---|---|
| Immunohistochemistry | 1:10-1:100 |
| Immunoblotting | 1:2000-1:10,000 |
| EIA | 1:5000-1:20,000 |




**Active aldehyde reaction scheme.**

**EZ-Link Plus Activated Peroxidase References**


Glover, L., *et al.* (2002). *Eur. J. Biochem.* **269**, 4607-4616.
Nawa, M., *et al.* (2000). *Clin. Diagn. Lab. Immunol.* **7**, 774-777.
Völkel, T., *et al.* (2001). *Protein Eng.* **14**, 815-823.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 31487 | EZ-Link Plus Activated Peroxidase (Periodate Activated) | 1mg |
| 31488 | EZ-Link Plus Activated Peroxidase (Periodate Activated) | 5 x 1mg |
| 31489 | EZ-Link Plus Activated Peroxidase (Periodate Activated) | Kit |
| | Includes: EZ-Link Plus Activated Peroxidase | 5 x 1mg |
| | Sodium Cyanoborohydride Solution | 1 x 0.5mL |
| | Quenching Buffer | 25mL |
| | BupH Phosphate Buffered Saline Pack | 500mL |
| | BupH Carbonate Buffer Pack | 500mL |

### Glutaraldehyde

Another method for conjugation uses glutaraldehyde, one of the oldest homobifunctional crosslinking reagents used for protein conjugation. It reacts with amine groups to create crosslinks by one of several routes. Under reducing conditions, the aldehydes on both ends of glutaraldehyde will couple with amines to form secondary amine linkages. The reagent is highly efficient at protein conjugation but has a tendency to form various high-molecular weight polymers, making results difficult to reproduce.

## EZ-Link Activated Peroxidase and Antibody Labeling Kit

*The easy way to make HRP-antibody conjugates.*

Primary amines on the Thermo Scientific EZ-Link Activated HRP have been converted to active aldehydes. This chemistry can be used to couple HRP to primary amines on another protein such as an antibody. The EZ-Link Activated Peroxidase Kit is designed to purify HRP-antibody conjugates by using Immobilized Protein A/G to separate unreacted enzyme from the antibody conjugate. Using EZ-Link Activated HRP produces an HRP-antibody conjugate in which both the enzyme activity and the antigen-binding activity are preserved.

### Highlights:

- Reacts with primary amines to form a covalent amide bond
- No reduction step is necessary to secure the linkage
- Can be stored for at least 12 months at –20°C
- One mg produces about 0.5mL of conjugate with a working dilution of 1:1000 when coupled to a high titer antibody
- Enzyme activity is > 200 units/mg

**EZ-Link Activated Peroxidase References**

Sandt, C.H. and Hill, C.W. (2001). *Infect. Immun.* **69**, 7293-7303.
Turpin, E.A., *et al.* (2003). *J. Clin. Microbiol.* **41**, 3579-3583.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **31496** | **EZ-Link Activated Peroxidase**<br>(Glutaraldehyde Activated) | 1mg |
| **31495** | **EZ-Link Activated Peroxidase**<br>(Glutaraldehyde Activated) | 5mg |
| **31497** | **EZ-Link Activated Peroxidase Antibody Labeling Kit**<br>(Glutaraldehyde Activated)<br>Includes: EZ-Link Activated Peroxidase<br>Conjugation Buffer<br>Lysine<br>Immobilized Protein A/G Column<br>Gentle Ag/Ab Binding Buffer<br>Gentle Ag/Ab Elution Buffer | Kit<br><br><br>5mg<br>50mL<br>250mg<br>0.5mL<br>200mL<br>200mL |

### Storing Enzyme Conjugates

A variety of Thermo Scientific reagents are available to help preserve enzyme conjugate activity. Typically, conjugates are aliquoted in 50-100µL increments using purified ethylene glycol (Product # 29810) as a preservative for –20°C storage. Conjugates can maintain activity for up to two years. An alternative to aliquoting is to use Thermo Scientific Pierce Peroxidase Conjugate Stabilizer (Product # 31503), diluting the conjugate 1:1 in the stabilizer and storing it at –20°C for up to one year as a stock solution. Pierce Peroxidase Stabilizer/Diluents (Product #s 37548 and 37552) allow peroxidase conjugates to be reconstituted and stored at 4°C as a 1:1000 dilution or a 1:100,000 dilution stock solution.

### Conjugate Stabilizers

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **37548** | **Pierce Peroxidase Conjugate Stabilizer/Diluent** | 200mL |
| **37552** | **Pierce Peroxidase Conjugate Stabilizer/Diluent** | 1L |
| **31503** | **Pierce Peroxidase Conjugate Stabilizer** | 25mL |
| **29810** | **Ethylene Glycol**<br>(50% aqueous solution) | 200mL |

# Choosing a Substrate

**We offer an extensive line of substrates for AP and HRP (Table 6). Which substrate to use depends upon the plate-reading equipment available and the level of sensitivity required in the ELISA.** Chemiluminescent and chemifluorescent substrates produce a more intense signal than colorimetric substrates, thus providing greater sensitivity. ELISA conditions must always be re-optimized when switching to a different substrate. Greater dilutions of a conjugate is used with a chemiluminescent substrate than with a colorimetric substrate.

Enzyme-labeled reagents are detected using chromogenic, chemiluminescent or chemifluorescent substrates. Chromogenic substrates are generally the least expensive, while luminescent or fluorescent substrates are often more sensitive. When performing ELISAs, a soluble substrate is used and converted to a soluble end product. ELISAs are performed on polystyrene plates, and the enzyme levels are determined by monitoring signal development with a spectrophotometer (a luminometer for luminescence or a fluorometer for fluorescence).

# Choosing a Substrate

**Table 6. Properties of Thermo Scientific ELISA Substrates for horseradish peroxidase (HRP), alkaline phosphatase (AP) and galactosidase (Gal).**

| Substrate | Product # | Page | Measurement / Color | Dilution range of Ab (from 1mg/mL stock) | Approximate Sensitivity* | Enzyme |
|---|---|---|---|---|---|---|
| SuperSignal ELISA Femto | 37075 | 62 | 425nm chemiluminescent | 1° 1:10K – 1:20K<br>2° 1:50K – 1:100K | 0.17pg/well | HRP |
| SuperSignal ELISA Pico | 37070 | 61 | 425nm chemiluminescent | 1° 1:1K<br>2° 1:10K | 0.5pg/well | HRP |
| QuantaBlu Substrate | 15169 | 63 | 325nm/420nm chemifluorescent | 1° 1:10K – 1:20K<br>2° 1:50K – 1:100K | 0.5pg/well | HRP |
| QuantaRed Substrate | 15159 | 64 | 560nm/590nm chemiluminescent | 1° 1:10K – 1:20K<br>2° 1:50K – 1:100K | 0.16pg/well | HRP |
| Pierce 1-Step Ultra TMB | 34028 | 66 | 450nm stopped – Yellow<br>652nm nonstop – Blue | 1° 1:500 – 1:1K<br>2° 1:4K – 1:100K | 2pg/well | HRP |
| Pierce 1-Step Turbo TMB | 34022 | 66 | 450nm stopped – Yellow<br>652nm nonstop – Blue | 1° 1:500 – 1:1K<br>2° 1:4K – 1:100K | 7pg/well | HRP |
| Pierce 1-Step Slow TMB | 34024 | 66 | 450nm stopped – Yellow<br>652nm nonstop – Blue | 1° 1:500 – 1:1K<br>2° 1:4K – 1:100K | 8pg/well | HRP |
| TMB Substrate Kit | 34021 | 66 | 450nm stopped – Yellow<br>652nm nonstop – Blue | 1° 1:500 – 1:1K<br>2° 1:4K – 1:100K | 5.5pg/well | HRP |
| Pierce 1-Step ABTS | 37615 | 65 | 410nm/650nm – Green | 1° 1:500 – 1:1K<br>2° 1:4K – 1:50K | 0.25ng/well | HRP |
| ABTS | 34062 | 65 | 410nm/650nm – Green | 1° 1:500 – 1:1K<br>2° 1:4K – 1:50K | 0.25ng/well | HRP |
| OPD Powder | 34005 | 65 | 410nm/650nm – Green<br>450nm nonstop – Yellow-Orange | 1° 1:500 – 1:1K<br>2° 1:4K – 1:100K | 7pg/well | HRP |
| OPD Tablets | 34006 | 65 | 410nm/650nm – Green<br>450nm nonstop – Yellow-Orange | 1° 1:500 – 1:1K<br>2° 1:4K – 1:100K | 7pg/well | HRP |
| Pierce 1-Step PNPP | 37621 | 67 | 405nm – Yellow | 1° 1:500<br>2° 1:5K | 10ng/well | AP |
| PNPP Kit | 37620 | 67 | 405nm – Yellow | 1° 1:500<br>2° 1:5K | 10ng/well | AP |
| PNPP Tablets | 34047 | 67 | 405nm – Yellow | 1° 1:500<br>2° 1:5K | 10ng/well | AP |
| PNPP Powder | 34045 | 67 | 405nm – Yellow | 1° 1:500<br>2° 1:5K | 10ng/well | AP |
| ONPG | 34055 | 68 | 410nm – Yellow | 1° 1:500<br>2° 1:5K | 10ng/well | -Gal |

** Actual sensitivity is unique to each antibody-antigen pair. The approximate sensitivities listed are conservative amounts that should be easily detectable for most antigens.*

**Choosing a detection signal type.**

| | Colorimetric Substrates | Chemifluorescent Substrates | Chemiluminescent Substrates |
|---|---|---|---|
| ELISA | • Medium/low sensitivity<br>• Generally less expensive<br>• Many substrates available<br>• Slow signal generation<br>• Enzyme catalyzed quickly<br>• Small linear range/poor low-end linearity<br>• Flexible (stopped, nonstopped and kinetic assays) | • High sensitivity<br>• Generally more expensive<br>• Few substrates available<br>• Rapid signal generation<br>• Enzyme activity maintained<br>• Large linear range/enhanced low-end linearity<br>• Flexible (stopped, nonstopped and kinetic assays) | • High sensitivity<br>• Generally more expensive<br>• Few substrates available<br>• Rapid signal generation<br>• Enzyme catalyzed quickly<br>• Large linear range/enhanced low-end linearity<br>• Nonflexible |
| Detection Equipment | Spectrophotometer | Fluorometer | Luminometer |

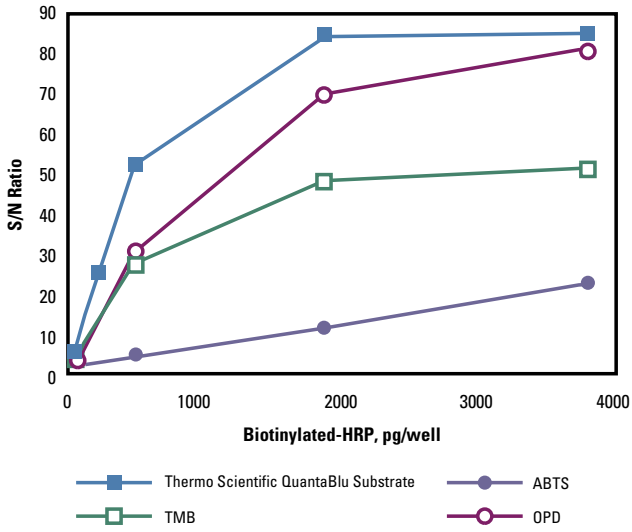
## Horseradish Peroxidase Substrates

Horseradish peroxidase is a 40kDa protein that catalyzes the oxidation of substrates by hydrogen peroxide, resulting in a colored or fluorescent product or the release of light as a byproduct. HRP functions optimally at a near-neutral pH and can be inhibited by cyanides, sulfides and azides. Antibody-HRP conjugates are superior to antibody-AP conjugates with respect to the specific activities of both the enzyme and antibody. In addition, a high turnover rate, stability, low cost and wide availability of substrates make HRP the enzyme of choice for most applications.

When selecting a substrate for HRP-based ELISAs, there are a number of possibilities. The question of sensitivity is often the overriding factor in making a selection. However, consideration should also be given to whether the substrate contains harmful solvents, what detection equipment is available and how the assay will be measured (kinetic or end-point). In many ELISA applications, colorimetric substrates provide a sufficient level of sensitivity and dynamic range. This is evident with our cytokine ELISA kits, in which a TMB substrate combined with a streptavidin-HRP detection system results in pg/mL sensitivity. Detection below this level requires a fluorescent or chemiluminescent signal. Characteristics of the HRP ELISA substrates we offer are summarized in Table 6.

Chromogenic ELISA substrates result in a soluble, colored product. These substrates are used in most ELISAs because detection of a colored product can be performed on a spectrophotometric plate reader. TMB (3,3′,5,5′-tetramethylbenzidine) is the most common chromogenic substrate for HRP and is available in several formats. Thermo Scientific Pierce 1-Step Ultra TMB (Product # 34028) yields the greatest sensitivity among the TMB substrates, followed by Pierce 1-Step Turbo TMB (Product # 34022) and Pierce 1-Step Slow TMB (Product # 34024). The Pierce 1-Step Substrates are preformulated so no mixing or pre-filtering is required. Although the sensitivity is lower, the Pierce 1-Step Slow TMB and Pierce 1-Step ABTS (2,2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) are ideal for kinetic readings. OPD (o-phenylenediamine dihydrochloride, Product # 34005) is another relatively sensitive HRP substrate that produces a yellow-orange color.

The greatest sensitivity in ELISA applications is obtained using chemiluminescent or chemifluorescent substrates. These substrates have been steadily gaining in popularity because of their sensitivity (less than 1pg/mL), large linear range for detection and excellent antibody conservation. We offer the chemiluminescent Thermo Scientific SuperSignal ELISA Pico (Product # 37070) and SuperSignal ELISA Femto (Product # 37075) Substrates and the chemifluorescent Thermo Scientific QuantaBlu Substrate (Product # 15169).




**Comparison of Thermo Scientific QuantaBlu Substrate to other substrates.** QuantaBlu Substrate and the colorimetric substrates were incubated for 30 minutes at room temperature (RT), followed by addition of a stop solution. QuantaBlu Substrate produced the greatest signal:noise (S/N) ratios and exhibited the lowest detection limit.

When energy in the form of light is released from a substance because of a chemical reaction, the process is called chemiluminescence. Luminol is one of the most widely used chemiluminescent reagents and its oxidation by peroxide results in creation of an excited state product called 3-aminophthalate. This product decays to a lower energy state by releasing photons of light (Figure 10).

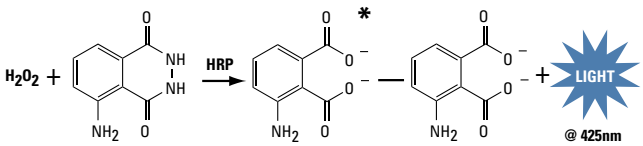



**Figure 10. Luminol is oxidized in the presence of horseradish peroxidase and hydrogen peroxide to form an excited state product (3-aminophthalate).** The 3-aminophthalate emits light at 425nm as it decays to the ground state.

# Choosing a Substrate

Chemiluminescent substrates have steadily gained in popularity throughout the past decade because they offer several advantages over other detection methods. Chemiluminescence has large linear response for detection and quantitation over a wide range of protein concentrations. Most importantly, chemiluminescence yields the greatest sensitivity of any available detection method. Using HRP as the enzyme label and Thermo Scientific SuperSignal ELISA Femto Chemiluminescent Substrate (Product # 37075), detection limits in the upper femtogram range are possible because the enhancers in this substrate greatly intensify the emitted light and extend the signal duration.

Chemiluminescent substrates differ from other substrates in that the signal is a transient product of the reaction that is only present while the enzyme-substrate reaction is occurring. This is in contrast to substrates that produce a stable, colored product; these colors persist in the well after the enzyme-substrate reaction has terminated. In a chemiluminescent ELISA, the substrate is the limiting reagent in the reaction; as it is exhausted, light production decreases and eventually ceases. A well-optimized procedure using the proper antibody dilutions will produce a stable output of light, producing consistent and sensitive results. When the antibody is not diluted sufficiently, too much enzyme is present and the substrate is used up quickly. A stable output of light will never be achieved. This is the single greatest cause of variability in chemiluminescent ELISAs. To avoid this problem, it is crucial to optimize the amount of antibody used for detection. Antibody suppliers typically suggest a dilution range for using their antibody in an ELISA. This dilution range is often appropriate for experiments detected with a relatively insensitive chromogenic substrate, but a much greater dilution is generally required for optimum performance with a sensitive chemiluminescent substrate.

**Advantages of enhanced chemiluminescence.**

| | |
|---|---|
| Sensitive | • Intense signal with low background<br>• Requires less antigen and antibody |
| Fast | • Rapid substrate processing<br>• Signal generated within seconds |
| Nonhazardous | • No health hazards<br>• No waste disposal problems |
| Stable | • Unlike radioisotopes, the shelf life is long<br>• Store at 4°C |
| Large linear response | • Can detect a wide range of protein concentrations |
| Quantitative | • Results are measured using a luminometer |

## HRP Substrates for ELISA

*Excellent sensitivity for use in luminometers.*

Chemiluminescent substrates are ideal for researchers looking for greater sensitivity in their ELISAs or any other solution-based assay. These ELISAs can take place in either a test tube or a microplate and are quantified by measuring relative light units (RLU) in a luminometer. Thermo Scientific SuperSignal ELISA Pico Chemiluminescent Substrate was developed for researchers who need high sensitivity at an economical price. SuperSignal ELISA Femto Maximum Sensitivity Chemiluminescent Substrate uses an enhancer system that meets the needs of high-throughput screening and diagnostics. Both have their own unique features as listed in Table 7.

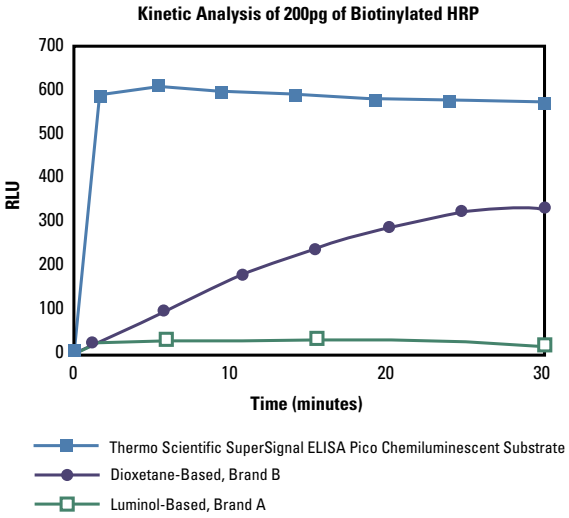
**Table 7. Which substrate is right for you?**

| Substrate | Detection Limits | Kinetics | Working Solution Stability at Room Temperature |
|---|---|---|---|
| Thermo Scientific SuperSignal ELISA Femto Maximum Sensitivity Substrate | femtogram | • Immediate light generation<br>• 5- to 30-minute stability, depending on HRP concentration | • 6-hour working solution stability |
| Thermo Scientific SuperSignal ELISA Pico Chemiluminescent Substrate | picogram | • Immediate light generation<br>• 30-minute stable light output | • 8-hour working solution stability<br>• Only 10% loss of activity after 24 hours |

## SuperSignal ELISA Pico Chemiluminescent Substrate

*Get high sensitivity and a large dynamic range.*

Thermo Scientific SuperSignal ELISA Pico Chemiluminescent Substrate is optimized for luminometer-based assays to generate an intense signal.




**Immediate generation of light with Thermo Scientific SuperSignal ELISA Pico Chemiluminescent Substrate.** Biotinylated HRP or biotinylated AP was added to separate wells of Thermo Scientific NeutrAvidin Coated White Polystyrene Plates. The plates were then incubated for 30 minutes at room temperature (RT) on a plate shaker and then each well was washed three times with BupH Tris-Buffered Saline. Working solutions of chemiluminescent substrates were prepared according to the manufacturers' instructions. For SuperSignal ELISA Pico Chemiluminescent Substrate and another luminol-based system (Brand A), 100µL of each substrate working solution was added to the appropriate plate well. For the dioxetane-based system, wells were washed with 1X Assay Buffer and 100µL of the dioxetane working solution was added to the appropriate plate well. All plates were incubated on a plate shaker at room temperature (RT) for 1 minute. Plates were then read on a plate luminometer with a 0.2 second read time per well. Several readings were taken over a 30-minute period.

### Highlights:

- Immediate generation of light – intense signal is produced immediately at room temperature or at 37°C
- High signal:noise ratio – minimal background
- Low picogram sensitivity – detect proteins in your ELISAs down to the picogram levels
- Room temperature storage – a consistent product with ambient shipping and no need to store at 4°C
- 8-hour working solution stability – consistent performance of the working solution over an 8-hour period with only a 10% decrease in activity at 24 hours
- Flexible – signal can be read in black or white opaque plates
- Emits light at 425nm


**References**

Bradley, K.A., *et al.* (2004). *J. Biol. Chem.* **278**, 49342-49347.
McKevitt, M., *et al.* (2003). *Genome Res.* **13**, 1665-1674.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **37070** | **SuperSignal ELISA Pico Chemiluminescent Substrate** | 100mL |
| | Includes: Luminol/Enhancer | 50mL |
| | Stable Peroxide Buffer | 50mL |

## SuperSignal ELISA Femto Maximum Sensitivity Substrates

*The most powerful substrate for high-throughput screening/ diagnostic applications with high sensitivity and superior low-end linearity.*

Thermo Scientific SuperSignal ELISA Femto Maximum Sensitivity Substrate is formulated for superior protein detection and low-end linearity in ELISA applications.

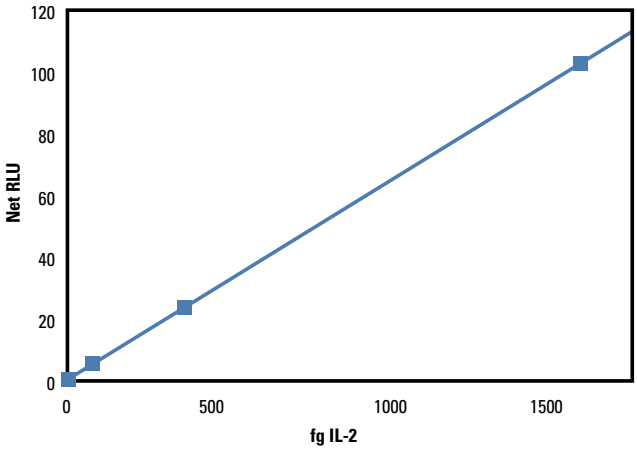
SuperSignal ELISA Femto Substrate's rapid signal generation has the added benefit of decreasing the substrate incubation period generating detectable light within 1 minute, saving up to 30 minutes per assay. This feature is ideal for high-throughput screening (HTS) applications in which as many as 100,000 assays may be run daily on robotic equipment. Because incubation periods are often a limiting factor in the development of rapid automated assays, SuperSignal ELISA Femto Substrate is the logical choice for automated HTS applications.

### Highlights:

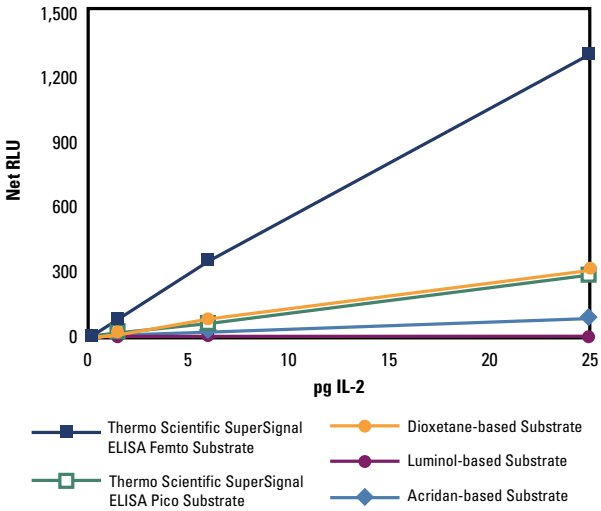
- Immediate light generation – intense signal generated immediately at both room temperature and 37°C
- Improved low-end linearity – easy detection of low quantities of proteins with high signal:noise ratios and low-end linearity of dose response curves
- High sensitivity – femtogram-level detection of target proteins
- Reduction in assay time – high sensitivity allows for reduction in incubation steps
- Stability – storage for six months at room temperature or a minimum of 12 months at 4°C with a six-hour working solution stability
- Emits light at 425nm

**Incubation time required to reach maximum signal.**

| | |
|---|---|
| **SuperSignal ELISA Femto Maximum Sensitivity Substrate** | 1 minute at room temperature |
| **SuperSignal ELISA Pico Chemiluminescent Substrate** | 1 minute at room temperature |
| **Dioxetane-based Substrate System** | 30 minutes at room temperature |
| **Luminol-based Substrate System** | 1 minute at room temperature |
| **Acridan-based Substrate System** | 5 minutes at room temperature |
| **TMB Colorimetric Substrate** | 15 minutes at room temperature |




**Femtogram detection of target protein and superior low-end linearity.** The dose response curve generated from an IL-2 ELISA illustrates the exceptional low-end linearity achieved with Thermo Scientific SuperSignal ELISA Femto Substrate and the incredible sensitivity attainable. ELISA, which was down to 168fg. The $R^2$ value of the curve was 1.00 for signal generated at less than 1600fg of IL-2.




**Signal intensity and kinetics comparison of chemiluminescent substrates.** The dose response curve using Thermo Scientific SuperSignal ELISA Femto Maximum Sensitivity Substrate in an IL-2 ELISA was compared to curves generated with a dioxetane-based substrate, another luminol-based substrate, an acridan-based system and TMB. SuperSignal ELISA Femto Maximum Sensitivity Substrate demonstrated immediate light generation with maximum peak intensity and high RLU values.

**References**

Brandt, E.B., *et al.* (2003). *J. Clin. Invest.* **112**, 1666-1677.
Hanley, N.R.S. and Hensler, J.G. (2002). *J. Pharmacol. Exp. Ther.* **300**, 468-477.
Masri, H.P. and Cornellissen, C.N. (2002). *Infect. Immun.* **70**, 732-740.
Su, S.V., *et al.* (2004). *J. Biol. Chem.* **279**, 19122-19132.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **37075** | **SuperSignal ELISA Femto Maximum Sensitivity Substrate** | 100mL |
| | Includes: Luminol/Enhancer | 50mL |
| | Stable Peroxide Buffer | 50mL |

## QuantaBlu Fluorogenic Peroxidase Substrates

*The ideal fluorescent substrates for use with peroxidase enzymes.*

A variety of substrates are available for detecting peroxidase activity in ELISA-based assays. Colorimetric substrates (e.g., TMB, OPD and ABTS) have been used widely for years. Each of these substrates varies greatly with respect to its performance characteristics such as detection sensitivity, working range and attainable signal:noise ratios. Substrate flexibility is also a key issue that affects an assay. Stability, development time requirements and the capacity to perform stopped and/or kinetic assays vary significantly among these substrates. Ideally, a substrate is stable, is very sensitive, has high attainable signal:noise ratios, possesses a broad dynamic range, and allows the user to perform stopped, nonstopped and kinetic assays. Thermo Scientific QuantaBlu Fluorogenic Peroxidase Substrate meets all the requirements of an ideal substrate for use in peroxidase detection.

QuantaBlu Substrate generates a blue fluorescent product upon reaction with peroxidase that does not photobleach. Fluorometric-based detection overcomes the limitations of colorimetric substrate detection, which does not allow for quantitation of greater than four optical density units. QuantaBlu Substrate allows for stopped, nonstopped and kinetic assays to be performed. Incubation times for stopped and nonstopped assays can be varied between 1 to 90 minutes at either room temperature or 37°C. QuantaBlu Substrate exhibits a flat baseline in assays, which facilitates low-level detection sensitivity and allows for high signal:noise ratios (see figure, top right).

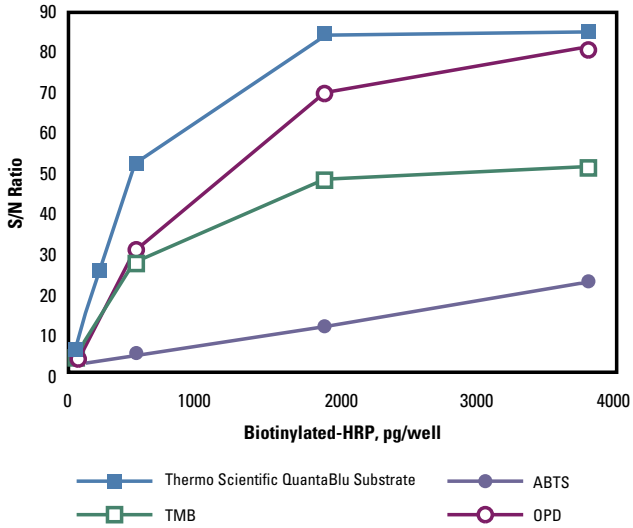
QuantaBlu Fluorogenic Peroxidase Substrate enables rapid detection of peroxidase at very low concentrations. Peroxidase is detected at 0-10pg per well from 1.5 to 6.5 minutes of substrate incubation time. At cycle 1 (1.5 minute incubation) as little as 2.5pg of peroxidase could be detected, while at cycle 6 (6.5 minute incubation) 0.625pg of peroxidase could be detected (see figure, lower right).

### Highlights:

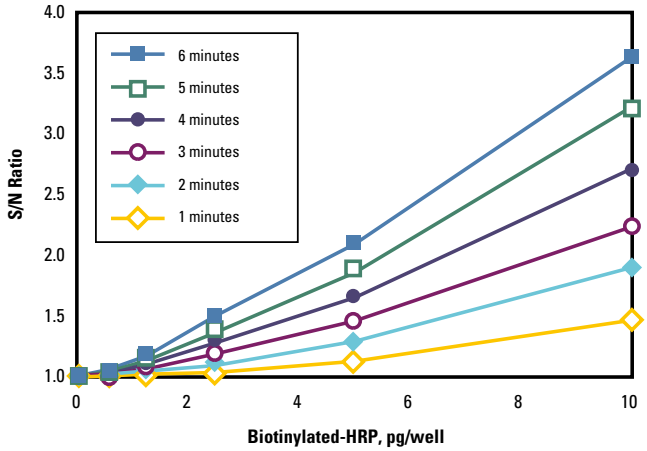
- More sensitive than TMB, OPD or ABTS substrates
- Flexible stopped, nonstopped or kinetic assays possible
- Large dynamic range (4 log peroxidase concentration range)
- Excellent stability – working solution is stable for 24 hours
- Large Stokes' shift; excitation/emission maxima of 325/420; range of 315-345/370-460
- Does not photobleach


**References**

Atamna, H., *et al.* (2000). *Proc. Natl. Acad. Sci.* **97**, 686-691.
Ayala, P., *et al.* (2002). *Infect. Immun.* **70**, 5965-5971.
Jefcoat, A.M., *et al.* (2001). *Am J Physiol Lung Cell Mol Physiol.* **281**, L704-712.
Savage, M.D., *et al.* (1998). *Previews* **2(1)**, 6-9.
Savage, M.D., *et al.* (1998). *Previews* **2(2)**, 18-19.


**Comparison of Thermo Scientific QuantaBlu Substrate to other substrates.** QuantaBlu Substrate and the colorimetric substrates were incubated for 30 minutes at room temperature, followed by addition of a stop solution. QuantaBlu Substrate produced the greatest signal:noise (S/N) ratios and exhibited the lowest detection limit.




**Rapid and sensitive detection with Thermo Scientific QuantaBlu Fluorogenic Peroxidase Substrate.** Bound biotinylated peroxidase was detected at 0-10pg/well. The assay was performed in a nonstopped mode using a 1-minute instrument cycle time between reads.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **15169** | **QuantaBlu Fluorogenic Peroxidase Substrate** | Kit |
| | Includes: QuantaBlu Substrate | 250mL |
| | QuantaBlu Stable Peroxide Solution | 30mL |
| | QuantaBlu Stop Solution | 275mL |
| **15162** | **QuantaBlu NS/K Substrate (for nonstopped and kinetic assays)** | Kit |
| | Includes: QuantaBlu Substrate | 250mL |
| | QuantaBlu Stable Peroxide Solution | 30mL |

## QuantaRed Chemifluorescent HRP Substrate

*Get the ultimate in sensitivity for ELISA-based applications.*

Thermo Scientific QuantaRed Enhanced Chemifluorescent HRP Substrate enables low picogram levels of detection in the ELISA format. The QuantaRed Substrate uses a proprietary technology to increase fluorescent yield and sensitivity of the well-documented ADHP (10-Acetyl-3,7-dihydroxyphenoxazine) chemifluorescence reaction. ADHP is a non-fluorescent compound that reacts with horseradish peroxidase (HRP) to produce resorufin, a soluble, highly fluorescent reaction product with excitation/emission maxima of ~570/585nm (Figure 11). The long wavelength emission of the resorufin minimizes interference from the low wavelength (blue and green) autofluorescence, making it an ideal substrate for quantitating target molecules in biological samples. In addition, the reaction product of QuantaRed Substrate is colorimetric as well as being fluorescent and can be read spectrophotometrically at ~570nm (Figure 11).

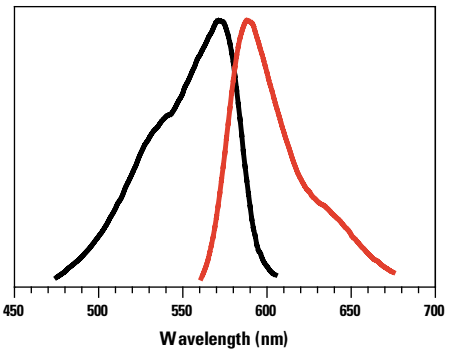



**Figure 11. Normalized absorption (black) and emission spectra (red) of the Thermo Scientific QuantaRed Enhanced Chemifluorescent HRP Substrate.**

Specially formulated, QuantaRed incorporates chemical enhancers for maximum sensitivity and superior low end linearity (Figure 11). With high quantum yield and large extinction coefficient, QuantaRed Enhanced HRP Substrate has sensitivity comparable to enhanced chemiluminescence and is more sensitive than the Amplex Red substrates for the detection of HRP in ELISA applications.

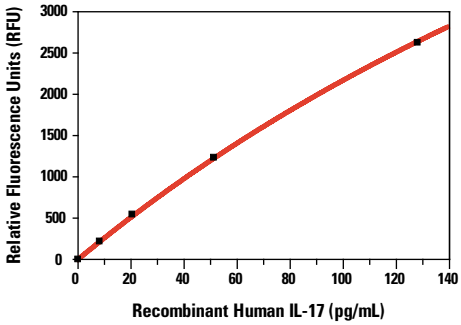



**Figure 12. Thermo Scientific QuantaRed Enhanced Chemifluorescent HRP Substrate enables low picogram target detection.** The detection limit for the assay was 1.6pg/mL (160fg) (Panel B). As determined by linear regression, PHA-stimulated PBMCs contained 16.5pg/mL human IL-17 and the unstimulated samples contained < 1.6pg/mL.

### Highlights:

- Sensitive – low picogram to femtogram detection
- Red-Shifted - minimal autofluorescence interference from biological samples
- Convenient – compatible with existing filter sets, no need to purchase special filter sets
- Complete – mix and use reagents, no buffers to prepare or reagents to dissolve
- Flexible – use Stop Solution to effectively stop the reaction resulting in stable signal for at least 4 hours or more (Figure 13)
- Versatile – produces a strong colorimetric signal and can be read fluorescently or colorimetrically at 570nm

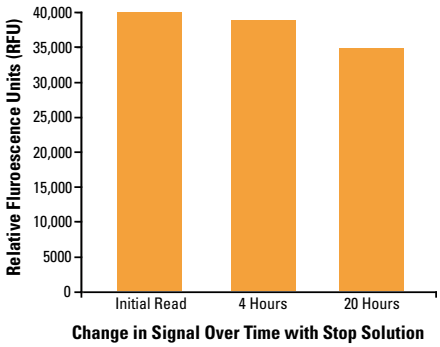



**Figure 13. Fluorescent signal is stable for several hours.** Four hours after adding the QuantaRed Stop Solution, the fluorescent signal was within 3% of the initial intensity. After 20 hours, the reaction product maintained nearly 87% of its original fluorescence.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 15159 | QuantaRed Enhanced Chemifluorescent HRP Substrate | Kit |
| | *Sufficient reagents to perform 10 x 96-well Assays* | |
| | Contains: ADHP Concentrate | 1mL |
| | Enhancer Solution | 50mL |
| | Stable Peroxide Solution | 50mL |
| | Stop Solution | 10mL |

## ABTS

ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]- diammonium salt) is a water-soluble HRP substrate that yields a green end product upon reaction with peroxidase. The green product has two major absorbance peaks, 405nm and 650nm. ABTS is less sensitive than OPD and TMB in ELISA applications. It is less readily oxidized, and its color development is slower (approximately 20 minutes). This may be advantageous if unacceptable background results from the use of the OPD or TMB substrates from higher sensitivities.

### Highlights:

- One component
- Ready-to-use
- Excellent choice when maximum sensitivities are not required
- Slow reaction that can be easily monitored with a kinetic reader


**References**

Paing, M.M., *et al.* (2002). *J. Biol. Chem.* **277**, 1292-1300.

Sau-Ching Wu, S.-C., *et al.* (2002). *Appl. Envir. Microbiol.* **68,** 3261-3269.


### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **34026** | ABTS | 50 tablets (10mg/ tablet) |
| **37615** | Pierce 1-Step ABTS | 250mL (Ready-to-use) |

## OPD

OPD yields a water-soluble yellow-orange product when reacted with peroxidase with an absorbance maximum of 492. OPD can easily be dissolved in a substrate buffer such as Thermo Scientific Stable Peroxide Substrate Buffer (Product # 34062).

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **34005** | OPD | 25g powder |
| **34006** | OPD Tablets | 50 tablets (5mg/ tablet) |

**Thermo Scientific Colorimetric Substrates for HRP.**

| Description | Highlights | Sensitivity | Kinetic Measurement Possible | Absorbance Maximum | Color | Product # |
|---|---|---|---|---|---|---|
| Pierce 1-Step ABTS | • **One component**<br>• Ready to use<br>• Excellent choice when maximum sensitivities are not required<br>• Slow reaction that can be easily followed with a kinetic reader | Low | Yes | 405nm | Green | 37615 |
| ABTS Tablets | | Low | Yes | 405nm | Green | 34026 |
| OPD Tablets | • OPD can be easily dissolved in a substrate buffer such as Stable Peroxide Substrate Buffer (10X) (Product # 34062) | High | No | 492nm | Yellow - orange | 34006 |
| OPD Powder | | High | No | 492nm | Yellow - orange | 34005 |
| TMB Substrate Kit | • High sensitivity<br>• Easy to use<br>• Results in seconds<br>• No DMF or DMSO in reagent | Very High | No | 450nm | Yellow | 34021 |
| Pierce 1-Step Ultra TMB-ELISA | • **One component**<br>• Ready to use<br>• Highest sensitivity<br>• No DMF or DMSO in the reagent | Very High | No | 450nm | Yellow | 34028 |
| Pierce 1-Step Turbo TMB | • **One component**<br>• Ready to use<br>• No DMF or DMSO in the reagent | High | No | 450nm | Yellow | 34022 |
| Pierce 1-Step Slow TMB | • **One component**<br>• Ready to use<br>• No DMF or DMSO in the reagent | Medium | Yes | 450nm | Yellow | 34024 |

# Choosing a Substrate

## TMB

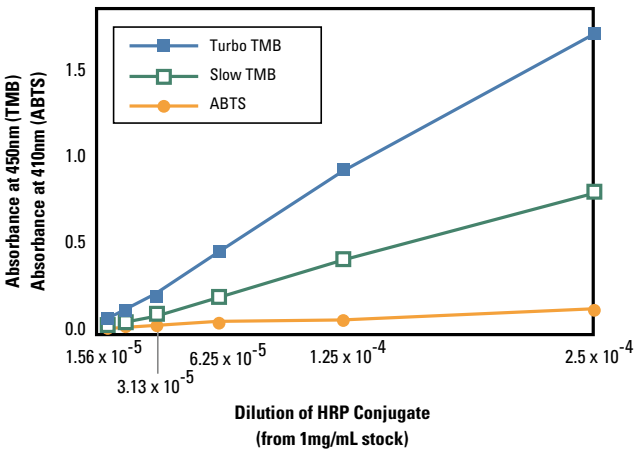
*The ideal fluorescent substrates for use with peroxidase enzymes.*

TMB is a chromogen that yields a blue color (measurable at 370nm or 652nm) when oxidized with hydrogen peroxide (catalyzed by HRP). The color then changes to yellow (measured at 450nm) upon addition of sulfuric or phosphoric acid to stop the reaction. TMB is very sensitive and more quickly oxidized than other HRP substrates, resulting in faster color development.

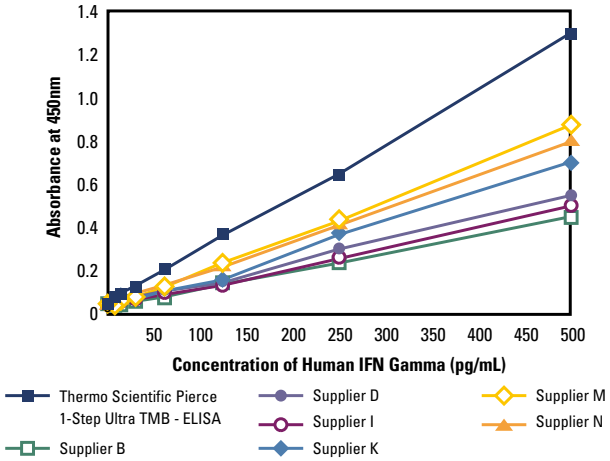
The Thermo Scientific 1-Step TMB Substrates are one-component substrates that require no preparation before use. Unlike other commercially available substrates, these products contain no DMF or DMSO. There are three formulations that differ primarily in their sensitivities. 1-Step Slow TMB is intermediate in sensitivity – ideal for kinetic readings. The sensitivity of the 1-Step Turbo TMB compares to that of OPD used at approximately 1mg/mL. 1-Step Ultra TMB-ELISA produces the highest signal:noise ratio and sensitivity in the picogram range, and has a three-year shelf life.

### Highlights:

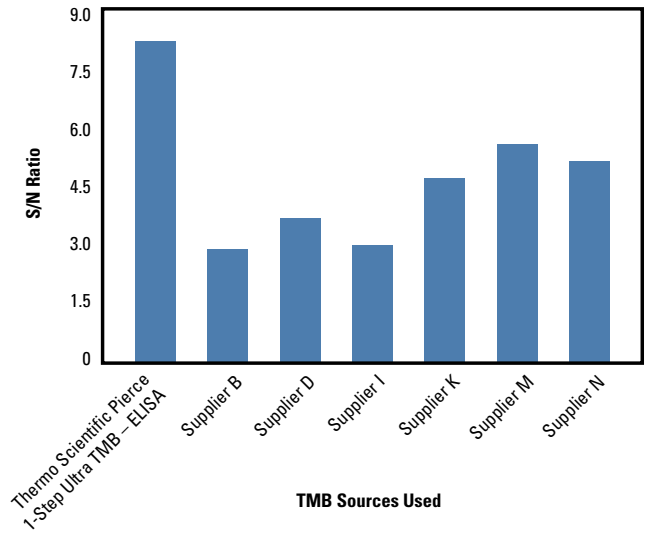
- Ready-to-use single component
- No hydrogen peroxide required
- No filtering required
- Noncarcinogenic
- Various sensitivities to suit any assay




**Comparison of sensitivities of Thermo Scientific Turbo TMB, Slow TMB and ABTS.**




**Thermo Scientific 1-Step Ultra TMB-ELISA provides more signal than other TMB substrates.**




**Thermo Scientific 1-Step Ultra TMB-ELISA produces higher signal: noise (S/N) ratios than other TMB substrates, and it has a three-year shelf life.**


**References**

Hong, P.W., *et al.* (2002). *J. Virol.* **76**, 12855-12865.
Murphy, M.B., *et al.* (2003). *Nucleic Acids Res.* **31**, e110.
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Tek, V. and Zolkiewski, M. (2002). *Protein Sci.* 11, 1192-1198.
Thomas, P.E., *et al.* (1976). *Anal. Biochem.* **75**, 168-176.
Weimer, B.C., *et al.* (2001). *Appl. Envir. Microbiol.* **67**, 1300-1307.
Wu, S.-C. and Wong, S.-L. (2002). *Appl. Envir. Microbiol.* **68**, 1102-1108.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 34024 | 1-Step Slow TMB | 250mL |
| 34022 | 1-Step Turbo TMB | 250mL |
| 34028 | 1-Step Ultra TMB-ELISA | 250mL |
| 34021 | TMB Substrate Kit<br>Includes: Peroxidase Substrate (TMB)<br>Peroxide Solution ($H_2O_2$) | Kit<br>200mL<br>200mL |

## Alkaline Phosphatase Substrates

Alkaline phosphatase (AP), a 140kDa protein that is generally isolated from calf intestine, catalyzes the hydrolysis of phosphate groups from a substrate molecule, resulting in a colored or fluorescent product or the release of light as a byproduct. AP has optimal enzymatic activity at a basic pH (pH 8-10) and can be inhibited by cyanides, arsenate, inorganic phosphate and divalent cation chelators, such as EDTA. As a label for ELISA, AP offers a distinct advantage over other enzymes. Because its reaction rate remains linear, detection sensitivity can be improved by simply allowing a reaction to proceed longer. Because of its large size, AP is sensitive to freeze/thaw cycles and should be aliquotted and stored at 4°C.

The most common ELISA substrate for alkaline phosphatase is the chromogen p-nitrophenyl phosphate (PNPP), which is available in several formats. PNPP yields a yellow reaction product that is water-soluble and absorbs light at 405nm. The Thermo Scientific Pierce 1-Step PNPP Substrate (Product # 37621) offers the convenience of a ready-to-use reagent with similar sensitivity to the two-component kit. The Phosphatase Substrate Kit (Product # 37620) includes PNPP tablets and diethanolamine buffer (5X). PNPP is also available as 25g of powder (Product # 34045) or in tablet form (Product # 34047). Diethanolamine Substrate Buffer (Product # 34064) is a convenient, ready-to-use formulation sold as a 5X concentrate with an optimal and consistent pH. Soluble ELISA substrates for AP are summarized in Table 6, on page 58.

**Thermo Scientific Pierce Alkaline Phosphatase Substrates.**

| Description | Sensitivity | Kinetic Measurement Possible | Absorbance Maximum | Color | Product # |
|---|---|---|---|---|---|
| 1-Step PNPP | High | Yes | 405nm | Yellow | 37621 |
| Phosphate Substrate Kit | High | Yes | 405nm | Yellow | 37620 |
| PNPP Tablets | High | Yes | 405nm | Yellow | 34047 |
| PNPP Powder | High | Yes | 405nm | Yellow | 34045 |

## PNPP

*Detection of alkaline phosphatase in ELISA applications.*

PNPP (p-nitrophenyl phosphate, disodium salt) is a widely used substrate for detecting alkaline phosphatase in ELISA applications.[1] When alkaline phosphatase and PNPP are reacted, a yellow water-soluble reaction product is formed. This product absorbs light at 405nm. We offer PNPP in four formats. PNPP is available either as a crystalline powder or 5mg tablets. Also available is the Thermo Scientific Phosphatase Substrate Kit, which contains PNPP tablets and a Diethanolamine Buffer to yield more than one liter of substrate. The Diethanolamine Substrate Buffer (Product # 34064) is also provided individually as a 5X concentrate. Our formulation has an optimal and consistent pH and it is stable even at a 1X concentration.

Until recently, the method for preparing PNPP solutions required dissolving the powder or tablets in buffer and then diluting the solution to the desired concentration. Substrate instability made it necessary to prepare it on a daily basis. The 1-Step PNPP circumvents this time-consuming and variable-introducing step by providing a single-component PNPP substrate. This substrate is stable for 12 months at 2-8°C and can be stopped with conventional methods.

### Highlights:

**Thermo Scientific Pierce 1-Step PNPP**

- One component
- Ready to use
- Stable for 12 months
- The easiest-to-use PNPP substrate available

**Phosphate Substrate Kit**

- Ideal for ELISA
- Easy to use
- Minimal assay-to-assay variability
- Low background


**References**

1. Snyder, S.L., *et al.* (1972). *Biochim. Biophys. Acta* **258**, 178-187.
Bosque, P.J., *et al.* (2002) *Proc. Natl. Acad. Sci.* **99**, 3812-3817.
Jan, J.-T., *et al.* (2000) *J. Virol.* **74**, 8680-8691.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 34045 | PNPP | 25g powder |
| 34047 | PNPP Tablets | 105 Tablets (5mg/tablet) |
| 37620 | Phosphate Substrate Kit<br>*Sufficient reagents for 1.05 liters of substrate.*<br>Includes: Diethanolamine Buffer<br>PNPP Tablets | Kit<br><br>225mL<br>105 tablets (5mg/tablet) |
| 37621 | Pierce 1-Step PNPP | 100mL |

## ONPG Colorimetric β-Galactosidase Soluble Substrate

*For detection of β-Galactosidase label in ELISA applications.*

When using β-Galactosidase as the label for proteins in ELISA studies, a wide variety of substrates are available, including o-nitrophenyl-β-D-galactopyranoside (ONPG), naphthol-AS-BI-β-D-galactopyranoside (Nap-Gal) and 4-Methyl-umbelliferyl-β-D-galactopyranoside (Mum-Gal). However, it is important to choose a substrate with adequate solubility, that uses readily available equipment and that gives a significant reading over the background. ONPG is a superior β-Galactosidase substrate option.[1] The product formed is completely soluble and has a high extinction coefficient at 405nm. The substrate yields a yellow product that is easily detectable in the visual range after stopping the reaction with 1M sodium carbonate.

### Highlights:

- Forms a completely soluble product when reacted with β-galactosidase
- High extinction coefficient at 405nm
- Yields a yellow product easily detectable in the visual range

**Characteristics of Thermo Scientific Pierce ONPG Substrate.**

| Description | Sensitivity | Kinetic Measurement Possible | Absorbance Maximum | Color | Product # |
|---|---|---|---|---|---|
| ONPG | High | Yes | 410nm | Yellow | 34055 |

**Reference**

1. Craven, G.R., *et al.* (1965). *J. Biol. Chem.* **240**, 2468-2477.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **34055** | ONPG | 5g powder |

## Substrate Buffers

*Substrate buffers for alkaline phosphatase and horseradish peroxidase.*

Buffered stable peroxide is used by researchers who prefer to make their own HRP-ELISA substrates. This is done easily by adding a chromogen of choice to the buffer. Thermo Scientific Stable Peroxide Substrate Buffer has a long shelf life (12 months after receipt), and is provided as a 10X concentrate. A total volume of 1000mL can be made from one 100mL bottle of concentrate. If you use 10mL of substrate per plate, you can make substrate for 100 plates from only one bottle of our Stable Peroxide Substrate Buffer. Our Diethanolamine Substrate Buffer is used with soluble alkaline phosphatase substrates such as PNPP. Our formulation is convenient, ready-to-use and reduces the possibility of assay-to-assay variability. Diethanolamine Substrate Buffer is sold as 5X concentrate with an optimal, consistent pH. It is stable even at a 1X concentration.

### Highlights:

- Ready-to-use
- Minimize assay-to-assay variability

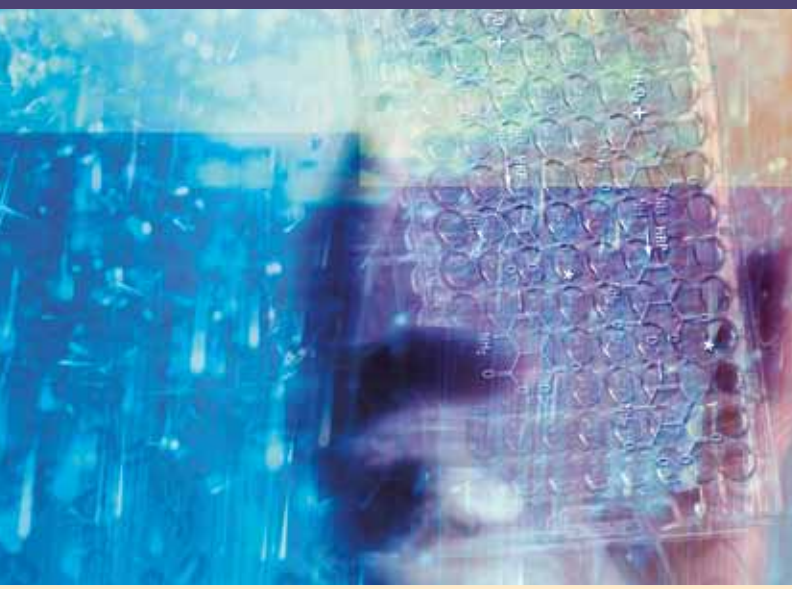
**References**

Louis, H., *et al.* (2000) *J. Histochem. Cytochem.* **48**, 499-508.
Neisewander, J.L., *et al.* (2000) *J. Neurosci.* **20**, 798-805.
Pifer, J., *et al.* (2002) *J. Immunol.* **169**, 1372-1378.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **34062** | Stable Peroxide Substrate Buffer (10X) | 100mL |
| **34064** | Diethanolamine Substrate Buffer (5X) | 225mL |
| **34047** | PNPP Tablets | 105 Tablets (5mg/tablet) |
| **37620** | Phosphate Substrate Kit<br>*Sufficient reagents for 1.05 liters of substrate.*<br>Includes: Diethanolamine Buffer<br>PNPP Tablets | Kit<br><br>225mL<br>105 tablets (5mg/tablet) |
| **37621** | 1-Step PNPP | 100mL |

# Bulk and Custom Offerings

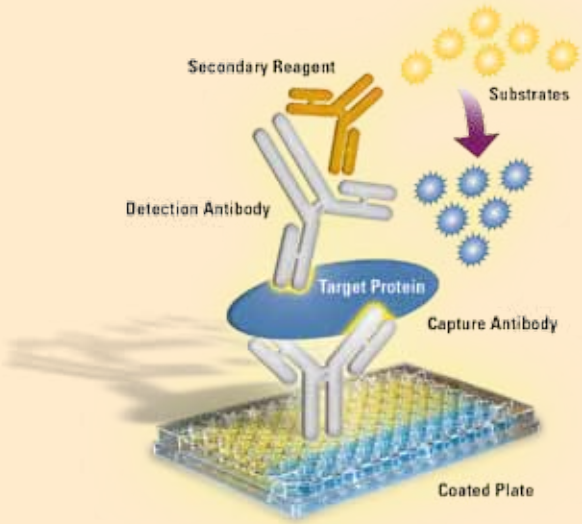


## Need more product than our catalog package size offers?

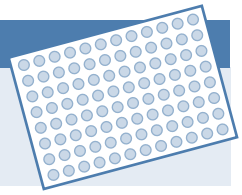
Partner with us to scale-up your process or product development pipeline with special pricing on bulk quantities of our high quality immunoassay reagents and biochemicals. We have been supplying bulk and custom made reagents to the biotechnology, diagnostic and pharmaceutical industry for over 50 years.

Contact our experts in the Bulk and Custom Sales Department or contact your local distributor to discuss your project or request a bulk pricing on our catalog products. Popular immunoassay reagents, protein purification supports, sample preparation devices, recombinant proteins and antibodies are supplied in bulk quantities. Custom microplate coating services are also available for assay product development (IVD) or screening (HTS) applications. We can also accommodate a variety of special packaging requests and formulations to accommodate your production schedule.

Our quality system is certified to ISO 9001 so manufactured products have validated manufacturing procedures and testing to maintain consistency. Rely on us to provide lot-to-lot consistency, QC methods development, multiple lot validation and large-scale lot production.




### COATED MICROPLATES



**(96-well or 384-well)**
Block unoccupied binding sites.

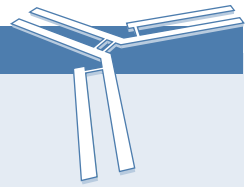
### BLOCKING BUFFERS

- Serum-based Blockers
- Milk-based Buffers
- Non-serum Blockers
- Stop Solutions

### IMMUNOASSAY BUFFERS

- Sample Preparation Buffers
- Assay Reagent Diluents
- Wash Buffers
- Stop Solutions

### IMMUNOASSAY CONJUGATES



- Antibody-enzyme Conjugates
- Biotinylated Antibody Conjugates
- Streptavidin-enzyme Conjugates
- NeutrAvidin-enzyme Conjugates
- Protein A, G, L or A/G-enzyme Conjugates
- Nickel-chelate Enzyme Conjugate

### ENZYME CONJUGATE STABILIZER

### SOLUBLE SUBSTRATES

- Colorimetric
- Fluorescent
- Chemiluminescent

# Bulk and Custom Offerings

## Custom Plate Coating Service

*The ligand of your choice…the plate of your choice… we'll turn them into the product you want!*

We have developed novel chemistries and coatings for a number of applications, and have the capacity to process up to 7500 microplates per day. Why not put our experience and expertise to work for you and develop the perfect combination of ligand, plate and blocker for your application. We will develop specific quality assurance tests to ensure we can provide the most consistent product to meet your needs and typically provide five test plates before we ship any lot of Thermo Scientific Pierce Custom Coated Plates to you. Get started with as few as 200 x 384-well or 500 x 96-well plates.

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We can provide the appropriate packaging for your custom plates, based upon your usage. For example, your plates can be packaged for large screening applications (e.g., 25 plates/pack ready for stacking) or for inclusion in a kit for resale (e.g., single-pouch packages).

**Plate Coating Options: Choose any plate, ligand and buffer combination.**

| Plate Type (96- or 384-well) | Coating Ligand | Blocking Buffer |
|---|---|---|
| Your Own Plate | Your Own Ligand | Your Own Blocker |
| Clear Plates | Antibodies | SuperBlock® Blocker |
| White Plates | Peptides | StartingBlock™ Blocker |
| Black Plates | Fusion Proteins | Protein-free Blocker |
| Clear Bottom, Black Plates | Metal Chelates | Purified Casein |
| Clear Bottom, White Plates | Biological Polymers | BSA |
| Filter Plates | Oligonucleotides | Serum |

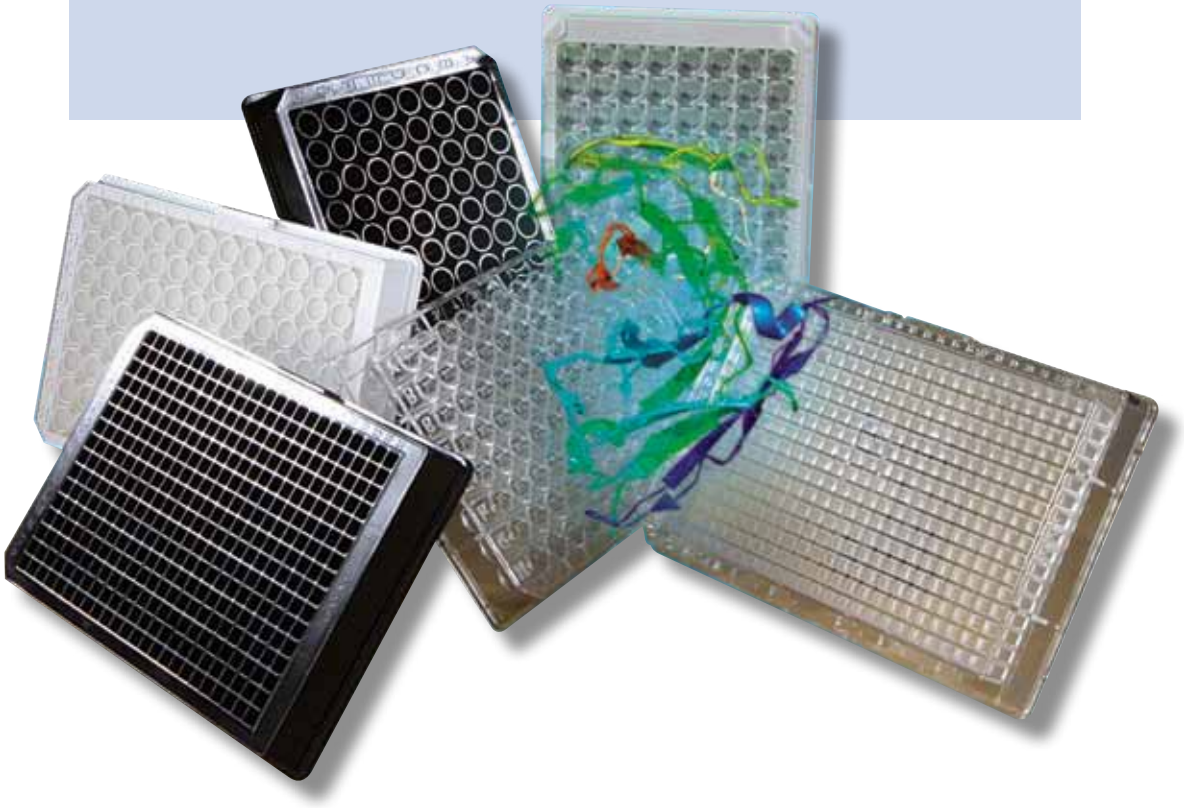
## Partner with us to get the coated plate of your choice!

**Do you need**

- Coated 96- or 384-well plates, slides or other coated surfaces?
- A coated plate using a certain type of plate or a specific supplier's plate?
- A specific surface chemistry that is not included in this catalog?

**We can help**

To learn about our plate-coating service, please contact us at Custom Large Volume & Custom Sales Department at 800-874-3723 or 815-968-0747, extension 300 or visit **www.thermoscientific.com/protein-custom**. Outside the U.S., contact your local distributor or branch office.



## Thermo Scientific Multiskan FC ELISA Reader

The Multiskan FC ELISA Reader delivers proven performance and reliable results for a wide variety of research and routine applications including:

- Immunoassays (ELISA)
- Protein assays
- Cytotoxity and cell proliferation
- Enzyme activity assay
- Growth curves
- Apoptosis
- Endotoxin

**Highlights:**

- Ease-of-use through multiple language versions
- Superior usability and logical workflow with Thermo Scientific SkanIt Software
- Reliability through built-in self-diagnostic and optional verification tools
- A broad wavelength range of 340 - 850nm for a wide variety of applications
- Fast reading of both 96- and 384-well plates

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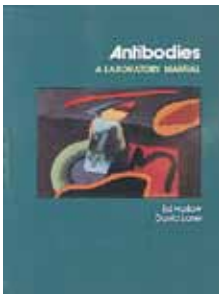
# Recommended Reading

## Using Antibodies: A Laboratory Manual




Harlow and Lane have completely revised their guide, Antibodies: A Laboratory Manual. Chapters have been entirely rewritten to provide background, context and step-by-step instructions for techniques ranging from choosing the right antibody and handling it correctly, to the proper methods for characterizing antigens in cells and solutions. They've also added new chapters on tagging proteins and epitope mapping. Rather than presenting an array of solutions for working with antibodies and antigens, Using Antibodies identifies the best approach to specific problems. These recommendations include more detail in the protocols, extensive advice on avoiding and solving problems, information regarding proper controls, and extensive illustration of theory, methods and results.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 15051 | Using Antibodies: A Laboratory Manual | 5g powder |

## Protein Assay Technical Handbook

The Protein Assay Technical Handbook is a helpful guide to choosing the best protein assay and standard for a sample. It contains abundant information on the most widely used protein assay methods (BCA, Lowry and Bradford Assays), including the principle behind each protein assay and its unique advantages and disadvantages. The handbook also includes methods for detection specific protein types such as histidine-tagged proteins, antibodies and proteases.

## Western Blotting Handbook and Troubleshooting Guide

This handbook contains full-length and abbreviated Western blotting protocols, tips on antibody and blocking buffer optimization, a chemiluminescent substrate selection guide, a procedure for stripping and reprobing Western blots, tips on clearer film development, and other information that will be useful to experts and first-timers alike. Then handbook also includes details on the new Pierce Fast Western line of Western blotting products.

## Antibody Production and Purification Technical Handbook

This handbook helps you choose the best methods to produce, purify, fragment and label antibodies. Topics include basic immunology, carrier proteins, adjuvants, antibody purification methods, antibody fragmentation with proteases, and labeling antibodies with a variety of tags (e.g., biotin, fluorophores, enzymes, iodine) for purification or detection.

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