Thermo Scientific
Pierce Cell Lysis Products
Technical Handbook Supplement

open to discovery

new reagents to extract and protect proteins

- neuronal protein extraction kits
- subcellular fractionation kits
- universal nuclease
- protease/phosphatase inhibitors

Thermo SCIENTIFIC

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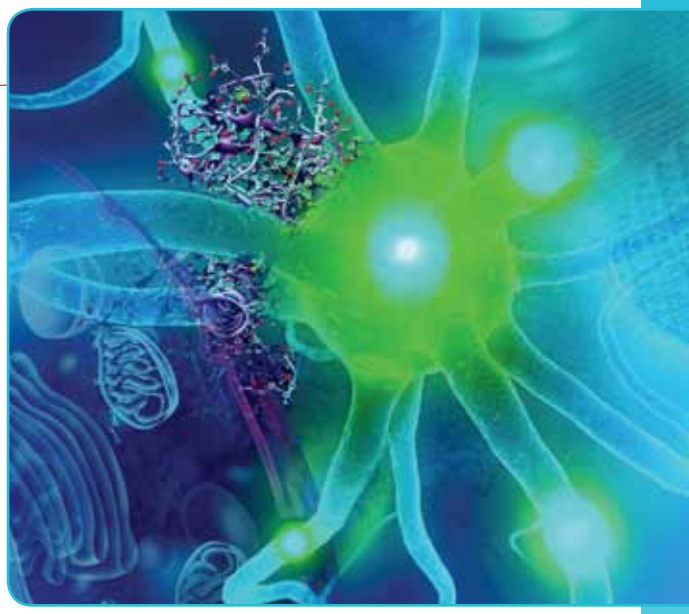
Cell Lysis Products Technical Handbook Supplement

easily isolate neuronal proteins



Neuronal tissue and primary cells are frequently used to study proteins involved in learning, behavior and neurodegenerative disease. The morphology and unique lipid composition of the neuronal cell membrane often make protein extraction from all cellular compartments inefficient. Dendrites branch near the neuronal cell body and act as signaling sensors, while the axon extends away from the cell body and transduces signals to the synapse. The insulative myelin sheath that coats axons is rich in glycolipid, sphingomyelin and cholesterol. The use of denaturing detergents overcomes this issue; however, their presence typically compromises protein function. With the introduction of the Thermo Scientific N-PER Neuronal Protein Extraction Reagent, a method now exists to easily isolate active neuronal proteins.

While many neuroscientists study intact neurons, a significant number focus their efforts on understanding the signal transmission events that occur at the neuronal cell-cell junctions, or synapses. Synaptic proteins can be enriched from synaptosomes, which are isolated nerve terminals generated during the homogenization of nerve tissue. Synaptosomes contain the complete presynaptic terminal, including mitochondria and synaptic vesicles, along with the postsynaptic membrane and the postsynaptic density (Figure 1). They are commonly used to study synaptic function because they contain functional ion channels, receptors, enzymes and proteins, as well as the intact molecular machinery for the release, uptake and storage of neurotransmitters. Preparation of synaptosomes typically involves several centrifugation steps to separate nerve termini from cell bodies and axons. Although synaptosomes are relatively easy to prepare in the laboratory, there has been no commercially available reagent for their efficient and consistent isolation until now. The proprietary Thermo Scientific Syn-PER Synaptic Protein Extraction Reagent efficiently isolates functional synaptosomes containing active synaptic proteins from neuronal tissue and primary cultured neurons.

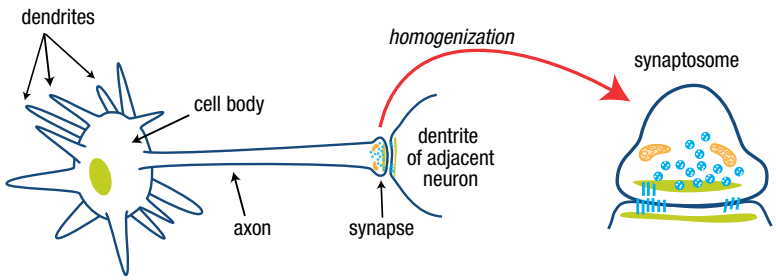


Figure 1. Basic structure of a neuron and a synaptosome containing detached nerve terminal with part of the postsynaptic membrane.

neuronal protein isolation

Optimized extraction reagent for neuronal tissue and primary cultured neurons

Thermo Scientific N-PER Neuronal Protein Extraction Reagent

N-PER™ Neuronal Protein Extraction Reagent is a specialized formulation for the extraction of total protein from neuronal tissue, providing higher yields and better extraction efficiency compared to other reagents while preserving protein function.

With the N-PER Lysis Reagent, protein extraction is completed in less than 30 minutes. For tissue samples, efficient extraction requires mechanical disruption (e.g., Dounce Homogenization, Polytron) in N-PER Reagent. Typical neuronal protein yields are 70-90µg of protein per mg of brain tissue or 300µg of total protein from 10^6 primary neurons. Neuronal cell lysates prepared with the N-PER Reagent may be used in downstream enzymatic activity assays (e.g., phosphatase, kinase, ATPase assays), immunoassays (e.g., Western blots, ELISAs, RIAs) and protein purification.

Highlights:

- **Optimized** – efficient extraction of total neuronal protein, including membrane proteins, from tissue or primary cultured cells
- **Gentle** – preserves protein function without compromising yield
- **Versatile** – can be supplemented with protease inhibitors, reducing or chelating agents or required cofactors
- **Compatible** – extracts are suitable for use with total protein, enzymatic and immunological assays and protein purification methods

High Efficiency Extraction of Proteins from Neuronal Tissue

N-PER Neuronal Protein Extraction Reagent extracts protein from neuronal tissues more efficiently than other extraction reagents tested, with 1.25- to 4-fold higher protein yields (Figure 1). Notably the proprietary formulation of N-PER Reagent increases extraction of integral membrane proteins (N-methyl-D-aspartate receptor type 2B) and membrane associated proteins (Flotillin-1 and Postsynaptic Density Protein 95) compared to other reagents (Figure 2).

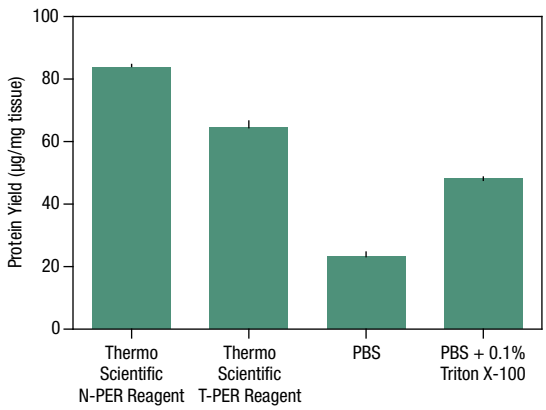


Figure 1. Increased protein yield per mg of neuronal tissue using Thermo Scientific N-PER Reagent. Yields (µg protein/mg tissue) of several extraction reagents are compared to N-PER Reagent using fresh mouse brain tissue and Dounce homogenization. All isolations were performed according to the supplied product instructions. Protein concentrations from cleared supernatants was determined using the Thermo Scientific BCA Protein Assay Kit (Product # 23225).

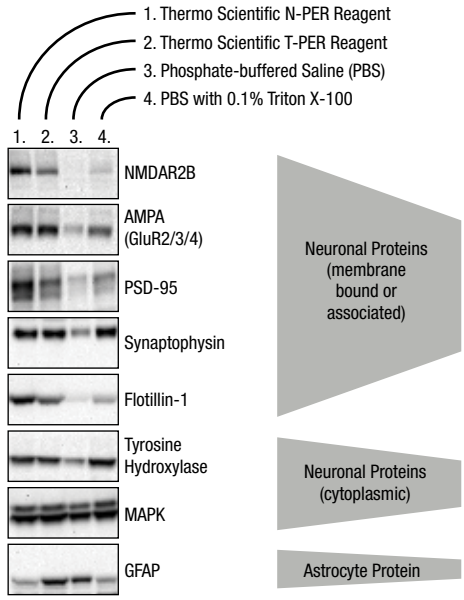


Figure 2. Increased extraction efficiency of specific neuronal proteins using Thermo Scientific N-PER Reagent. Lysates (10µL per well) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with antibodies for specific neuronal proteins, including membrane bound/associated proteins. Blots were developed with Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34077). **Lane 1:** N-PER Neuronal Protein Extraction Reagent, **Lane 2:** T-PER® Tissue Protein Extraction Reagent, **Lane 3:** Phosphate-buffered saline, **Lane 4:** Phosphate-buffered saline with 0.1% Triton® X-100.

Isolation of Active Neuronal Protein using N-PER Reagent

To assess protein function after extraction with N-PER Reagent, we performed several activity assays related to neuronal function. The Rho GTPases (including Rho, Rac, and Cdc42) regulate actin and mictrotubule dynamics, with Rac1 promoting neurite formation and Rho serving as an inhibitor. We evaluated the ability of N-PER Reagent to extract native Rho and Rac GTPases from fresh mouse brain. Lysates were supplemented with 5mM $MgCl_2$ and either 10µM GTPγS or 10µM GDP to keep the native Rho or Rac protein in active and inactive forms, respectively. Active Rho or Rac was isolated from the treated lysates using the Thermo Scientific Active Rho Pull-down and Detection Kit (Product # 16116) or Active Rac Pull-down and Detection Kit (Product # 16118). Both proteins were able to bind GTPγS or GDP in their respective nucleotide binding sites, and the GTP-bound form was capable of interacting with its downstream effector in the pull-down assay (Figure 3), indicating that the function of Rho and Rac1 was maintained.

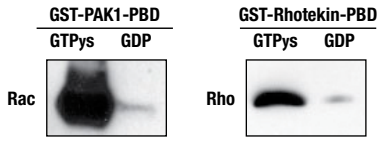


Figure 3. Thermo Scientific N-PER Reagent does not affect activity of GTPases isolated from neuronal tissue. N-PER Reagent was used to isolate brain tissue lysates. Lysates(1mg) were supplemented with 5mM $MgCl_2$ and treated with GTPγS or GDP were incubated with the indicated GST-PBD and glutathione resin. Active Rho and Rac were isolated following protocols for Active Rho Pull-down and Detection Kit (Product # 16116) and Active Rac Pull-down and Detection Kit (Product # 16118). Half of the eluted sample volumes were analyzed by Western blot and probed using small GTPase-specific antibodies provided in the respective kits.

To determine if protein phosphatases remain active upon extraction, we measured fluorescence of Fluorescein Diphosphate (FDP), a substrate that fluoresces after phosphate cleavage. Phosphorylation of molecules in the brain is important for many neurophysiological events, such as neurotransmitter release, long-term potentiation and neuronal differentiation. In particular, protein tyrosine phosphatases (PTP1B, SHP-2, PTEN and LAR) and serine/threonine phosphatases (PP1 and PP2A) are involved in cell signaling and are classes of enzymes targeted for therapeutic drug development. Although there was not a dramatic difference in phosphatase activity between N-PER Reagent and Thermo Scientific T-PER Tissue Protein Extraction Reagent, both reagents preserved enzymatic activity (Figure 4). Similarly, ATPase/kinase activity is maintained (Figure 5), indicating that researchers could use this reagent for screening small molecules that may affect kinase activity.

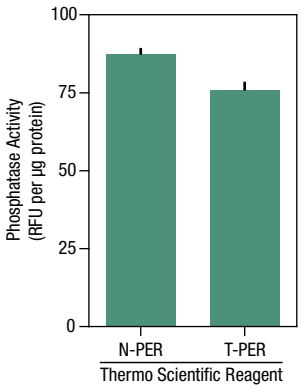


Figure 4. Thermo Scientific N-PER Reagent preserves protein phosphatase activity. Brain tissue lysate (10µg) produced using N-PER Reagent was incubated with a fluorogenic phosphatase substrate for 1 hour at 37°C. The change in fluorescence (excitation=485nm, emission=520nm) due to substrate hydrolysis was measured using the Thermo Scientific Varioskan Flash Multimode Plate Reader.

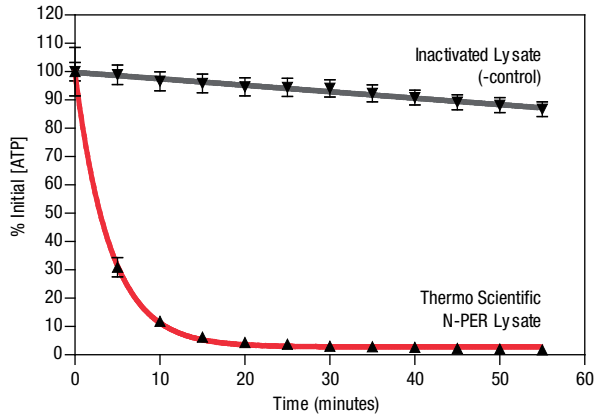


Figure 5. Thermo Scientific N-PER Reagent preserves kinase/ATPase activity. ATPase activity was measured as a function of luciferase chemiluminescence. Brain tissue lysates (10µg) produced using N-PER Reagent were spiked with 5mM ATP. A portion of the lysate was inactivated as a negative control. Lysates were then added to an equal volume of a luciferase-based reaction mix and luminescence was measured with a Varioskan Flash Multimode Plate Reader at 5 minute intervals over the course of 1 hour. Values were normalized to initial luminescence values.

To further evaluate neuronal membrane protein function in N-PER lysates, we measured the protein activity of β-secretase1 (BACE1, β-site APP cleaving enzyme 1), a transmembrane aspartyl protease responsible for the cleavage of amyloid precursor protein (APP), which is associated with beta-amyloid (Aβ) creation. Beta-amyloid peptide is potentially involved in cell signaling, protection from oxidative stress and transcription. Accumulation of Aβ in the brain is also associated with Alzheimer's disease. Beta-secretase and gamma secretase are important in the progression of Alzheimer's disease through the increased generation of Aβ-peptide insoluble aggregates from the miscleavage of amyloid precursor protein. N-PER Reagent solubilizes BACE1 from the membrane, and preserves activity (Figure 6). Brain tissue processed with the N-PER Reagent may be a useful sample source in β-secretase small molecule inhibitor screens.

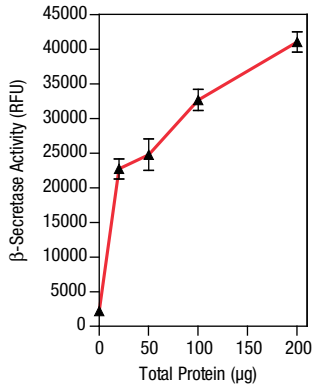


Figure 6. Thermo Scientific N-PER Reagent solubilizes active β-Secretase, an integral membrane protease involved in the generation of amyloid-β peptide. Activity of β-secretase was evaluated using a FRET-based assay system (EMD Millipore). Increasing concentrations of brain tissue lysate produced using N-PER Reagent were added to wells containing a fluorogenic peptide substrate, and incubated for 1 hour at 37°C. Fluorescence was measured at an excitation wavelength of 345nm and an emission of 495nm using a Safire Plate Reader.

Ordering Information

Product #	Description	Pkg. Size
87792	N-PER Neuronal Protein Extraction Reagent Sufficient for 10g tissue or up to 200 x 10cm dishes of primary cultured neurons	100mL

neuronal protein isolation

Isolate functional synaptosomes, extract synaptic proteins, and maintain phosphoprotein integrity

Thermo Scientific Syn-PER Reagent

The proprietary Syn-PER™ Synaptic Protein Isolation Reagent efficiently isolates functional synaptosomes containing active synaptic proteins from neuronal tissue. In addition, Syn-PER Reagent facilitates the study of labile or transient neuronal protein phosphorylation events by stabilizing or preserving these modifications during tissue disruption.

The protocol used with Syn-PER Reagent takes approximately one hour from the start of brain tissue homogenization until collection of the synaptosomal suspension when processing ten samples or less. Syn-PER Synaptic Protein Extraction Reagent efficiently enriches pre-and post-synaptic protein with high yield. The nondenaturing cell lysis reagent is compatible with many downstream applications, including neurotransmitter release assays, enzyme assays (e.g., phosphatase, kinase), immunoassays, various chromatography procedures and electrophoresis. In addition, Syn-PER Reagent preserves phosphoprotein integrity better than most commercially available extraction buffers, even in the absence of phosphatase inhibitors. However, inhibitors such as Thermo Scientific Protease and/or Phosphatase Inhibitor Liquid Cocktails or Tablets can be added just before use to prevent proteolysis or to offer additional protection from the high phosphatase activity normally present in brain tissue.

Highlights:

- **Efficient extraction** – obtain up to 10µg of synaptic protein per milligram of neuronal tissue or 4µg synaptic protein per 35mm dish of primary cultured neurons (10^6 cells)
- **Gentle formulation** – isolate viable synaptosomes, extract native synaptic proteins and preserve phosphoprotein integrity
- **Fast procedure** – obtain synaptosomal suspension in less than one hour
- **Simple protocol** – requires no ultracentrifugation steps

Efficient synaptic protein extraction and enrichment

We compared the total synaptic protein yields of samples prepared with Syn-PER Reagent or a standard homemade buffer obtained from the literature using a general dounce extraction protocol for fresh mouse brain tissues (Figure 1). The synaptic protein yield in samples obtained using Syn-PER Reagent was about three-fold higher compared to samples prepared with homemade buffer (Figure 2). The total protein concentration of the synaptosome suspension prepared with Syn-PER Reagent was 9.7 ± 1.0µg/mg brain tissue, while with the homemade buffer, we obtained a yield of 3.4 ± 0.8µg/mg brain tissue.

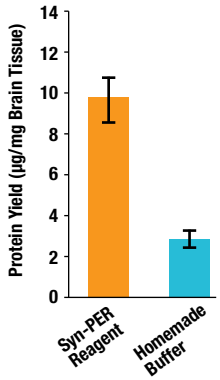
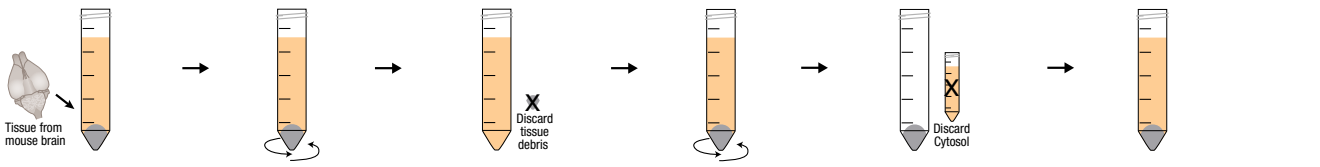


Figure 2. Comparison of protein yield from synaptosome suspension prepared with Thermo Scientific Syn-PER Reagent and homemade buffer. Whole brain or one hemisphere excluding the cerebellums (about 200-400 mg) was homogenized as one sample in 10 volumes of Syn-PER Reagent or homemade buffer (protease inhibitors included; Product # 87785) using a 7mL Dounce tissue. The homogenate was centrifuged and supernatant collected. The supernatant was further centrifuged and the pellets, containing synaptosomes, were gently resuspended in their respective buffer. Protein content was estimated using Thermo Scientific BCA Protein Assay Kit (Product # 23225).

To determine the specificity of the synaptosome extraction procedure, we performed Western blot analyses to identify individual synaptic proteins and overall synaptic protein enrichment. The immunoreactivity of N-methyl-D-aspartate receptor 2B subunit (NMDAR2B), PSD95, GluR2/3/4 of α-amino-3hydroxy-5-methyl-4-ioxazolepropionic acid (AMPA) receptor, and synaptophysin in the homogenate, cytosol, and synaptosome suspension was determined (Figure 3). In samples prepared with Syn-PER Reagent, the immunoreactivity of each synaptic protein in the synaptosome suspension is substantially enriched compared to that of the initial homogenate. However, in samples prepared with homemade buffer, little to no enrichment was observed as the signal in the synaptosome suspension is comparable to that of the homogenate. The purity of the synaptosome suspension was further confirmed by probing for the cytosolic proteins, calcineurin and CDK5, and nuclear marker protein HDAC2, which are not significantly represented in the synaptosome fraction, as well as the ubiquitously-expressed protein β-actin as a loading control.



1. Add 10mL of Syn-PER Reagent per gram of tissue. Homogenize at 4°C.
2. Centrifuge at 1200 x *g* for 10 minutes at 4°C.
3. Discard tissue debris pellet and transfer supernatant to a new tube.
4. Centrifuge supernatant at 15,000 x *g* for 20 minutes at 4°C.
5. Remove supernatant (cytosolic fraction) from synaptosome pellet.
6. Suspend synaptosome pellet in 1-2mL of Syn-PER Reagent for downstream analysis. Maintain at 4°C (-20°C for long-term storage).

Figure 1. Protocol for the isolation of synaptosomes from mouse brain using Thermo Scientific Syn-PER Reagent or homemade buffer.

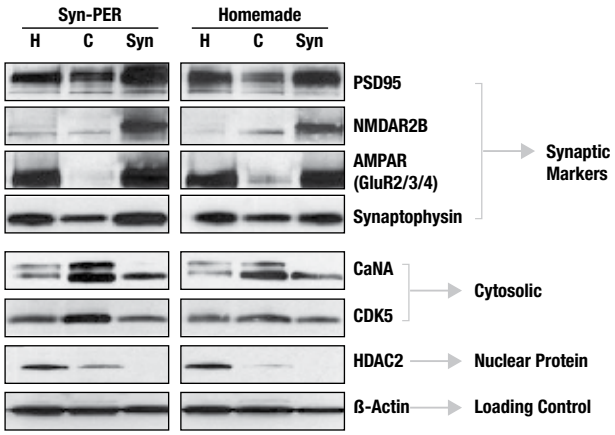


Figure 3. Improved enrichment of pre- and postsynaptic marker proteins is obtained in samples prepared using Thermo Scientific Syn-PER Reagent compared to homemade buffer. Ten micrograms total protein from homogenates (H), cytosol (C), and synaptosome suspension (Syn) were analyzed by Western blotting using antibodies against specific pre- and postsynaptic marker proteins including synaptophysin, PSD95, NMDA receptor 2B subunit, AMPA receptors (GluR2/3/4), as well as Calcineurin (A (CaNA), Cdk5, and β-Actin as purity and loading controls. The blots were probed with goat anti-rabbit HRP or goat anti-mouse HRP and detected with Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34077).

Functional synaptosome isolation

To determine if synaptosomes prepared with Syn-PER Reagent are functional, we measured synaptic vesicle endocytosis and exocytosis by monitoring the uptake and release of FM2-10, a lipophilic styryl fluorescent dye[1]. When the styryl dye FM2-10 was incubated with the synaptosome suspension prepared with Syn-PER Reagent, the endocytotic vesicles internalized FM20-10, resulting in a detectable fluorescent intensity measured at Ex506/Em620 nm (Figure 4). In the presence of calcium, KCl stimulation induced the release of accumulated FM2-10 into solution where FM2-10 is virtually nonfluorescent, and a slow decay of FM2-10 fluorescent intensity over 18 minutes was detected. This result indicates that the Syn-PER- prepared synaptosome suspension is capable of uptake and release of fluorescent dye FM2-10. Because both endocytosis and exocytosis are highly controlled biological processes regulated by a variety of synaptic proteins, the results of the FM2-10 uptake and release assay also demonstrates that proteins in the Syn-PER-prepared synaptosome suspension are functional. Therefore, Syn-PER isolated synaptosomes provide a useful model with which to study endocytosis and exocytosis in synaptic vesicles, as well as synaptic transmission.

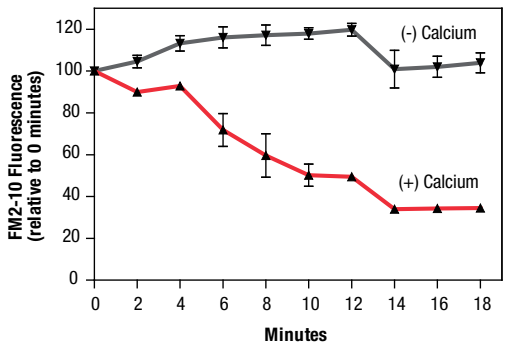


Figure 4. Ca^{2+}-dependent and KCl-evoked release of FM2-10 in synaptosomes prepared using Thermo Scientific Syn-PER Reagent. Synaptosomes were resuspended in HBSS either plus or minus 1.2 mM $CaCl_2$. The suspensions were then incubated with 100µM FM2-10 for 15 minutes. The release of FM2-10 was induced by the addition of 30mM KCl. Release of accumulated FM2-10 was then monitored at Ex506/Em620 nm as a decrease in fluorescent intensity upon release of the dye into solution where FM2-10 is no longer fluorescent. Arrow indicates the time point of adding depolarizing agent 30 mM KCl. Each point is the mean ± SD of two samples.

Phosphoprotein preservation significantly improved

To compare protein phosphorylation levels between samples prepared with Syn-PER Reagent and commercial lysis buffers, antibodies specifically recognizing phospho-ERK (Thr202/Tyr204), phospho-GluR2 (Try869/Tyr873/Try876) of AMPA receptor, and phospho-PSD95 (Tyr236/Tyr240) were used. Western blots detected higher immunoreactivity for each phosphorylated protein in fresh mouse brain homogenates (Figure 5A) and synaptosome suspensions (Figure 5B) prepared with Syn-PER Reagent than in those homogenates prepared with commercial detergent-containing lysis buffers 1 and 2. The levels of total ERK immunoreactivity were comparable among samples prepared with Syn-PER Reagent and both commercial lysis buffers. These data suggest that protein phosphorylation levels are significantly better preserved when brain tissues are homogenized and synaptosome suspensions are prepared using Syn-PER Reagent.

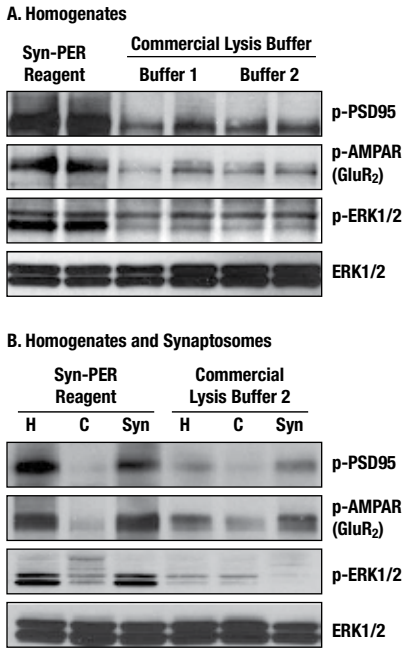


Figure 5. Thermo Scientific Syn-PER Reagent provides better preservation of phosphoprotein immunoreactivity than other commercial lysis buffers. Western blot comparison of immunoreactivity of phosphoproteins, p-PSD95, p-GluR2 of AMPA receptor, and p-ERK1/2, between samples prepared with Syn-PER Reagent and commercial lysis buffers in homogenates **(H) (Panel A)** and both homogenates, cytosol fraction **(C)** and synaptosome suspension **(Syn) (Panel B)**. Equal amounts of total protein (10–20µg/lane) were resolved on denaturing 2–10% SDS-polyacrylamide gels. Western blots were performed with the appropriate antibodies and bands were visualized using Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34080).

References

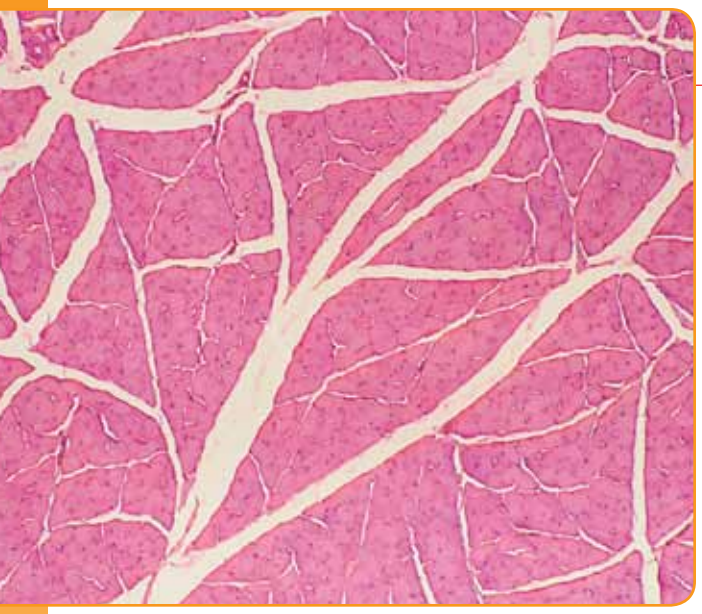
1. Baldwin, M.L., *et al.* (2003). Two modes of exocytosis from synaptosomes are differentially regulated by protein phosphatase types 2A and 2B. *J Neurochem.* **85:**1190-9.
2. Bai F and Witzmann. (2007). Synaptosome proteomics. *Subcell Biochem.* **43**:77–98.
3. Salter M.W., *et al.* (2009). Regulation of NMDA receptors by kinases and phosphatases. *Biology of the NMDA Receptor.* **7**:123-48

Ordering Information

Product #	Description	Pkg. Size
87793	**Syn-PER Synaptic Protein Extraction Reagent** Sufficient for 10g tissue or 500 x 35mm dishes of primary cultured neurons	100mL

subcellular fractionation

Fractionate subcellular proteins from a variety of tissue types



quickly and efficiently extract protein from tissue

Proteins within a cell are localized to specific cellular compartments that provide crucial information about the function of the protein. The study of protein localization and translocation is increasingly important for understanding protein function, disease and cancer. Subcellular protein fractionation provides a means to assess protein localization, enrich low-abundance proteins, reduce sample complexity for proteomics, and monitor physiologic fluxes and redistribution under basal and stimulated/diseased conditions.

Protein translocation is often assessed through isolation of specific cellular compartments using differential ultracentrifugation or by sequential extractions of cellular components. As research trends from cell culture systems to animal models, the extraction of cellular components from tissue samples with minimal contamination from other compartments will become increasingly important. Isolation of proteins from tissue samples presents an additional level of complexity with the tightly woven network of proteins and extracellular matrices. The Thermo Scientific Subcellular Protein Fractionation Kit for Tissue employs differential detergents as a fractionation alternative that results in the sequential extraction of five cellular compartments from one tissue sample, while minimizing cross-contamination.

Thermo Scientific Subcellular Protein Fractionation Kit for Tissue

The Subcellular Protein Fractionation Kit for Tissue was specifically developed to address the unique structures present in many different tissue types, such as heart, kidney, brain, liver, spleen and lung. This complete kit allows the fractionation of five cellular compartments (cytoplasm, membrane, nuclear, chromatin-bound and cytoskeleton) from one single tissue sample without the use of ultracentrifugation. The extracts obtained are functional and compatible with many downstream applications such as protein assays, Western blotting, gel-shift assays and enzyme-activity assays.

The supplied buffers have been formulated to efficiently extract protein from 50-200mg of tissue, with minimal contamination between compartments. A tissue strainer has been added to remove tissue debris after homogenization, and buffer extraction volumes have been optimized for effective protein concentrations in each fraction.

The Subcellular Protein Fractionation Kit for Tissue employs differential detergents as a fractionation alternative that results in the sequential extraction of five cellular compartments from one tissue sample. The tissue sample is homogenized in the first reagent, which causes selective membrane permeabilization, releasing soluble cytoplasmic contents (Figure 1). This homogenate is transferred to a tissue strainer to remove excess tissue debris. The second reagent dissolves plasma, mitochondria and ER-golgi membranes, but does not solubilize the nuclear membranes. After recovering intact nuclei by centrifugation, a third reagent yields the soluble nuclear extract. An additional nuclear extraction with micrococcal nuclease is performed to release chromatin-bound nuclear proteins. The recovered insoluble pellet is then extracted with the final reagent to isolate cytoskeletal proteins.

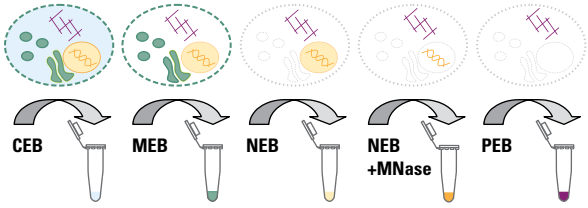


Figure 1. Schematic of the Thermo Scientific Subcellular Protein Fractionation Kit procedure.

Highlights:

- **Optimized** – formulations and protocols specific for fractionation of tissue
- **Complete** – obtain cytoplasmic, membrane, soluble nuclear, chromatin-bound and cytoskeletal protein fractions from a single kit
- **Time efficient** – extract functional protein fractions without ultracentrifugation in approximately 2 hours
- **Convenient** – unique tissue strainer allows rapid removal of tissue debris
- **Compatible** – use extracts for downstream applications such as protein assays, Western blotting, gel-shift assays and enzyme-activity assays
- **Versatile** – extract proteins from a variety of tissue types, including liver, heart, brain, kidney, lung and spleen

Effectively fractionate functional subcellular proteins from a variety of tissue types

To evaluate fractionation efficiency, the Subcellular Protein Fractionation Kit for Tissue was used with six different mouse tissue types including heart, brain, kidney, lung, liver and spleen. Protein localization was assessed for each fraction by Western blotting and probed with compartment-specific protein markers to show minimal cross-contamination between the fractions (Figure 2).

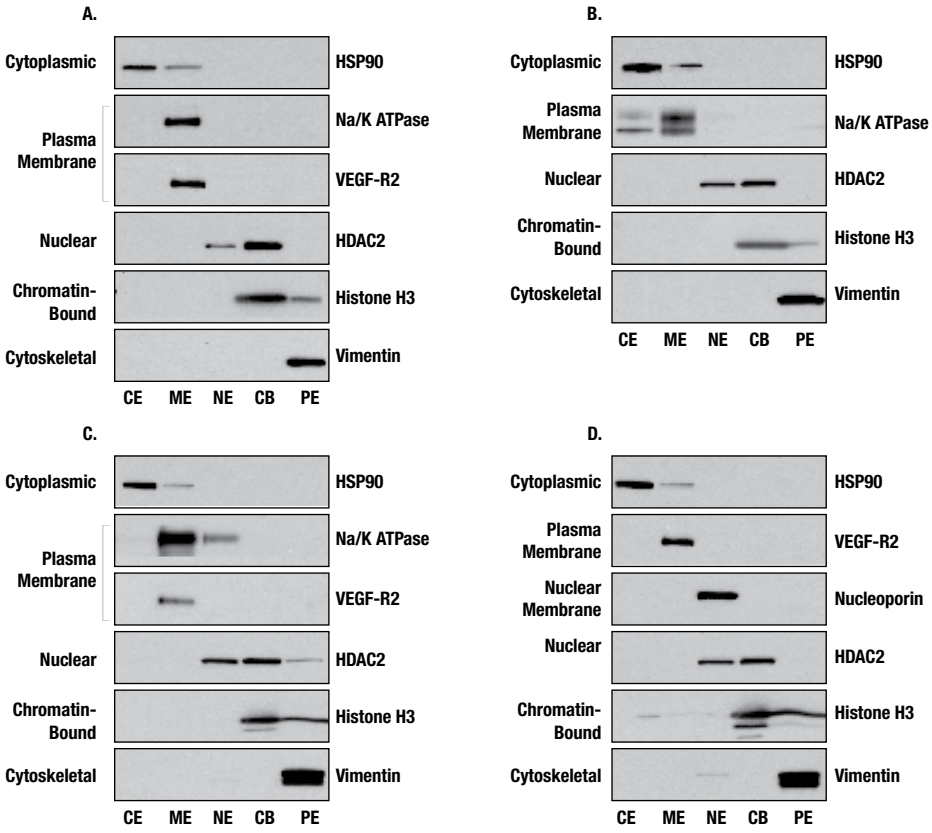


Figure 2. Thermo Scientific Subcellular Protein Fractionation Kit for Tissue efficiently isolates compartmentalized proteins. Mouse tissue samples were fractionated using the Subcellular Protein Fractionation Kit for Tissue according to instructions provided. Normalized loads of each extract (10µg) were analyzed by Western blotting. Subcellular protein fractionation of mouse **A.** heart (125mg) **B.** brain (175mg) **C.** kidney (200mg) **D.** Lung (100mg). **CE:** cytoplasmic extract, **ME:** membrane extract, **NE:** nuclear extract, **CB:** chromatin-bound extract, **PE:** pellet extract.

subcellular fractionation

Easy recovery with tools to facilitate sample processing

To demonstrate protein functionality after subcellular fractionation, nuclear extracts from mouse brain tissue were used in an electrophoretic mobility shift assay (EMSA). The Thermo Scientific LightShift Chemiluminescent DNA EMSA Kit was used to successfully perform an EMSA using biotin-labeled DNA sequences known to bind to the transcription factor activator protein-1 (AP-1) (Figure 3). This heterodimeric transcription factor regulates gene expression in response to a variety of stimuli including infections, stress, growth factors, and cytokines.

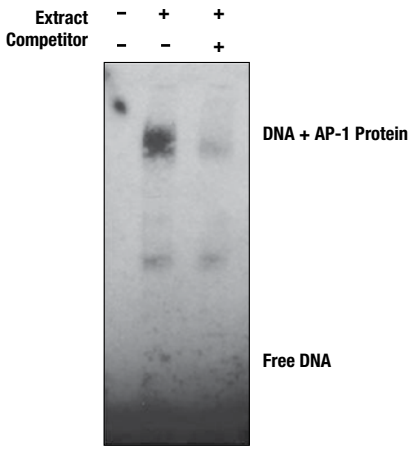


Figure 3. EMSA using mouse brain nuclear extract. Mouse brain (180mg) was fractionated using the Subcellular Protein Fractionation Kit for Tissue. The resulting nuclear extract was used to perform an EMSA using the Thermo Scientific LightShift EMSA Kit. Biotin-labeled and competitor (non-labeled) DNA duplexes were used to perform the reactions.

Ordering Information

Product #	Description	Pkg. Size
87790	Protein Subcellular Fractionation Kit for Tissue *Sufficient for 25 extractions of 200mg tissue each* Includes: Cytoplasmic Extraction Buffer (CEB) for Tissues	Kit 50mL
	Membrane Extraction Buffer (MEB) for Tissues	35mL
	Nuclear Extraction Buffer (NEB) for Tissues	20mL
	Pellet Extraction Buffer (PEB) for Tissues	6.5mL
	100mM $CaCl_2$	450µL
	Micrococcal Nuclease, 100 units/µL	260µL
	Halt Protease Inhibitor Cocktail (100X)	1mL
	Pierce Tissue Strainers, 250µm	25 each

Thermo Scientific Pierce Tissue Strainer

The Pierce Tissue Strainer can be used to quickly separate cells or cell lysates from tissue debris following mechanical disruption. The debris is separated by passing homogenates through a 250µm mesh filter using gravity or gentle centrifugation. The easy-to-use Pierce Tissue Strainers are made of strong nylon and fit into standard 15mL conical tubes, making the processing and recovery of samples simple and reliable. The strainers can be filled with 2.5mL of cells or lysate at one time although multiple loads are possible, with stepwise sample application.

The Pierce Tissue Stainers can be used with the Subcellular Protein Fractionation Kit for Tissue and other protein extraction reagent protocols that utilize tissue samples. Removal of large tissue debris facilitates easier sample handling and can protect downstream instrumentation and devices by eliminating fouling of injection ports and columns.

Ordering Information

Product #	Description	Pkg. Size
87791	Pierce Tissue Strainers 250µm, 2.5mL Sufficient for 50 separations, 2.5mL per load of tissue homogenate	50 units

Highlights:

- **Convenient** – remove debris from tissue homogenate within minutes using gravity filtration or centrifugation
- **Compatible** – unique design fits into standard 15mL conical tubes
- **Versatile** – remove debris from a variety of tissues types, including liver, heart, brain, kidney, lung and spleen

Protect your proteins with new inhibitor tablets

convenient tablets

protect your extracted proteins

Proteases and phosphatases serve many metabolic and regulatory functions in the cell. However, upon cellular lysis, the once compartmentally-contained enzymes are capable of mass degradation and dephosphorylation. The preservation of proteins from protease and phosphatase degradation after cell or tissue lysis is essential for many downstream research applications. For this reason, it is common practice to add multiple compounds known to inactivate or inhibit proteases and phosphatases to cell lysis buffers or cell extracts.

Proteases are a ubiquitous class of enzymes that hydrolyze the peptide bonds in proteins. Proteases are divided into two broad categories, based on cleavage site, and then further subdivided into additional categories based on the active site and pH preferences. The exopeptidases (carboxypeptidases and aminopeptidases) cleave peptides proximal to the amino or carboxy termini of the substrate, while endopeptidases (serine, aspartic, cysteine, and metalloproteinases) cleave the peptide distal to the terminus. Plants contain the largest distribution of proteases (44%), followed by bacteria (18%), fungi (15%), animals (11%), algae (7%), and viruses (4%). However, the variety, distribution and compartmentalization of proteases vary between the different organisms.

Phosphatases are hydrolase enzymes that remove phosphates from proteins and other molecules. Phosphatases are categorized based on sequence, structure and catalytic function. Protein phosphatases generally target serine, threonine and tyrosine phosphorylation on proteins during cell signaling. The balance of phosphorylation/dephosphorylation by protein phosphatases and kinases is vital to many cellular signaling pathways, including signal transduction, cell division and apoptosis. Acid phosphatases are localized in lysosomes and operate optimally in the acidic conditions of the lysosomes while alkaline phosphatases prefer a more basic pH. The phosphatases are also ubiquitous, but depending on function, are concentrated in certain cells types and tissues.

protease/phosphatase inhibitors

Protect your proteins with new inhibitor tablets

Thermo Scientific Pierce Protease and Phosphatase Inhibitor Tablets

Thermo Scientific Pierce Protease, Phosphatase and combination Protease and Phosphatase Inhibitor Tablets have been formulated for broad-spectrum protection from the major classes of proteases and phosphatases. These formulations (Table 1) have been validated using multiple cell and tissue extracts with a variety of protease and phosphatase substrates. Of note, the combined tablet is the only tablet on the market that contains both protease and phosphatase inhibitors. The tablets are provided in perforated blister packs for easy reconstitution directly in the lysis buffer before extract preparation for maximum protection.

Highlights:

- **Two formulations** – available with or without EDTA
- **Complete** – each tablet contains a broad spectrum of protease and/or phosphatase inhibitors
- **Easy-to-use** – tablets are easily removed from perforated blister packs and disperse readily into solution
- **Compatible** – use with cell lysis reagents prepared by your lab or commercially-available

To assess the inhibitor activity of the Pierce Protease Inhibitor Tablet formulations (Product # 88660; Table 1), inhibitor tablets were reconstituted and incubated with fluorescent protease substrates and mouse pancreatic extract. Cleavage of the protease substrates results in fluorescence that can be monitored at 460nm. Our Pierce Protease and Phosphatase Inhibitor Tablet formulations (Product # 88663; Table 1) were tested similarly. The inhibition activity was also compared to other commercial formulations. Results suggest that the protease tablet formulation almost completely inhibited papain activity (97% and 94%-EDTA-free), and performed favorably when compared to other commercial formulations (Figure 1). Similarly, the protease tablets were able to inhibit greater than 90% of papain activity in different tissues lysates (Figure 2). The combined protease and phosphatase formulation inhibited papain by 86% (Figure 1). Currently, the combined tablets have no commercial comparisons.

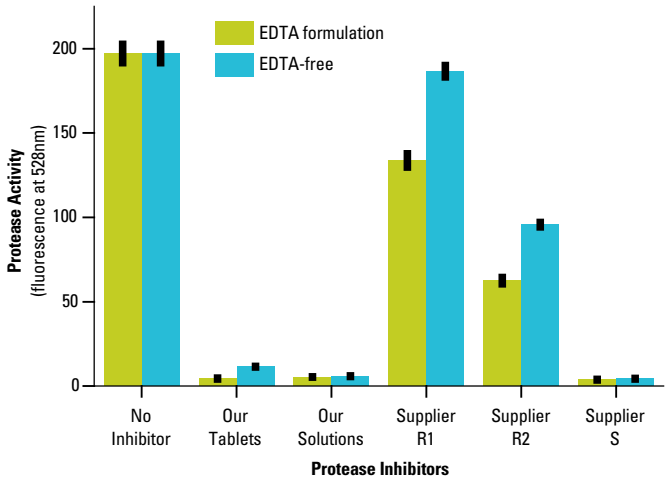


Figure 1. Comparison of commercially available protease inhibitor tablets and cocktails. Pancreatic extract was incubated with a quenched-fluorescent protease cleavable substrate in the presence or absence of commercially available protease inhibitors with EDTA-containing or EDTA-free formulations. Reactions were incubated for 2 hours at 37°C and the fluorescence determined.

Table 1. Formulation of Thermo Scientific Pierce Protease and Phosphatase Inhibitor Tablets.

Component	Inhibitor	Target	Protease Product #s 88660, 88661	Phosphatase Product # 88662	Combined Product #s 88663, 88664
AEBSF•HCl	Protease	Serine proteases/irreversible	X		
Aprotinin	Protease	Serine protease/reversible	X		X
Bestatin	Protease	Aminopeptidase/reversible	X		X
E-64	Protease	Cysteine/irreversible	X		X
Leupeptin	Protease	Serine and cysteine protease/reversible	X		X
Pepstatin	Protease	Aspartic acid proteases/reversible	X		
EDTA (not in 88661 or 88664)	Protease	Metallo-proteases/reversible (chelates divalent cations)	X		X
Sodium fluoride	Phosphatase	Serine/threonine and acidic phosphatases		X	X
Sodium orthovanadate	Phosphatase	Tyrosine and alkaline phosphatases		X	X
β–glycerophosphate	Phosphatase	Serine/threonine phosphatase		X	X
Sodium pyrophosphate	Phosphatase	Serine/threonine phosphatase		X	X

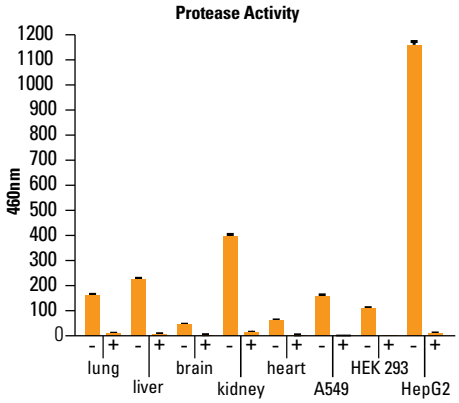


Figure 2. Inhibitor activity of Thermo Scientific Pierce Protease Inhibitor Tablets in different tissue lysates. Fresh tissue was homogenized in Thermo Scientific T-PER Reagent and clarified. Protease activity of lysates (1mg/mL) was determined in the presence or absence of protease inhibitor at 1X.

Phosphatase activity was assessed using assays for acid, alkaline and protein phosphatase assays as well as measurement of phosphorylation preservation by Western blotting. The Pierce Phosphatase Inhibitor Tablets (Product # 88662; Table 1) and Pierce Protease and Phosphatase Inhibitor Tablet formulation (Product # 88663; Table 1) were reconstituted to 2X and incubated with mouse brain extract and fluorescent phosphatase substrates. Cleavage of the phosphate results in fluorescence that can be monitored at 528nm. When compared to other formulations, our Phosphatase Inhibitor Tablets were able to inhibit protein phosphatases 91%, acid phosphatases 71%, and alkaline phosphatases 95% (Figure 3). The combined tablets were able to prevent greater than 90% of acid phosphatase activity (Figure 3).

To test preservation of phosphorylation during cell signaling, NIH 3T3 cells were stimulated with PDGF after serum-starvation. Preservation of serine/threonine and tyrosine phosphorylation was assessed by Western blotting using AKT and PDGF as phosphorylation targets, Figure 4). Lysis of the cells in the presence of the inhibitors is beneficial as it preserves phosphorylation of the targets.

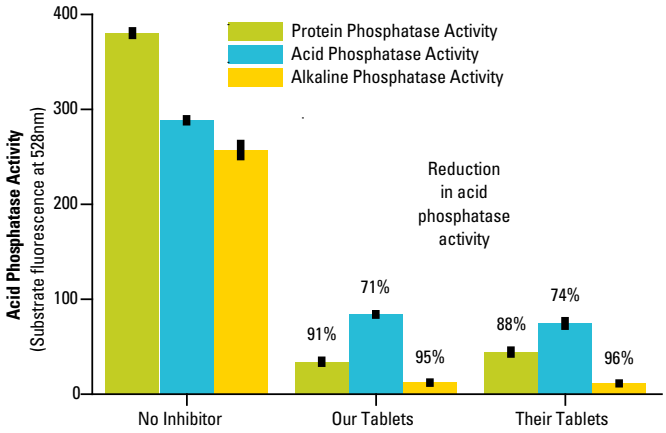


Figure 3. Thermo Scientific Pierce Phosphatase Inhibitor Tablets preserve phosphorylation in lysates. Fluorescent acid, alkaline or protein phosphatase substrate (MFP, FDP, and FDP, respectively) was coated onto black plates, followed by addition of protease inhibitor (1X final) and brain extract (diluted in assay sample buffer to 0.4mg/mL). Activity was assessed in the presence and absence of the indicated inhibitor formulations. Plates were incubated for 1 hour at 37°C. and activity was measured using a Thermo Scientific VarioSkan Flash Plate Reader (ex: 485nm; em: 528nm).

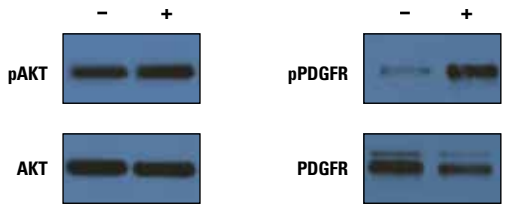


Figure 4. Preservation of serine/threonine and tyrosine phosphorylation using Thermo Scientific Pierce Phosphatse Inhibitor Tablets. Western blots of targets were assayed after BNIH3T3 cells were stimulated with PDGF. For each condition, 20mg of protein lysate was used for detection of AKT, pAKT, PDGF, and pPDGF.

References:

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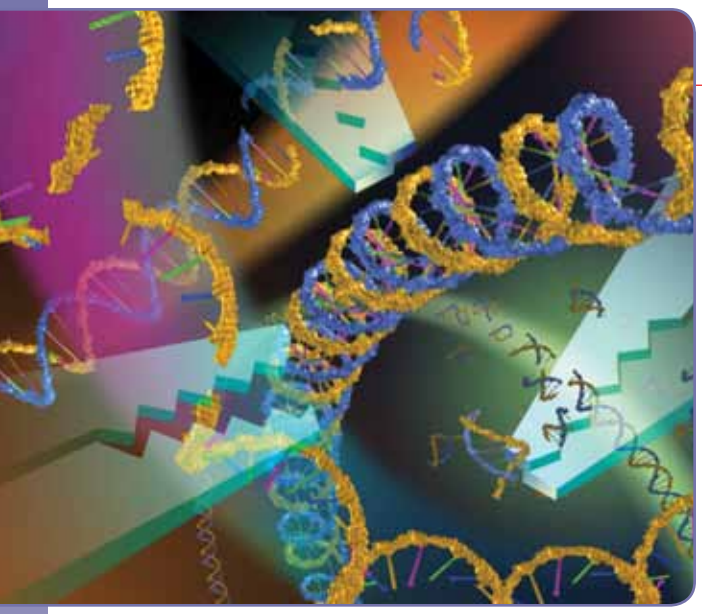
2. Bull, H., Murray, P.G., Thomas, D., Fraser, A. M. and P.N. Nelson. (2002). Demystified...Acid Phosphatases. *J. Clin Pathol. Mol Pathol.* **55**:65-72.
3. Stoker, A.W. (2005). Protein tyrosine phosphatases and signaling. *J. Endocrinology* **185**:19-33.
4. Millan, J. L. (2006). Alkaline Phosphatases: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. *Purinergic Signalling* **2**:335-341.

Ordering Information

Product #	Description	Pkg. Size
88660	**Pierce Protease Inhibitor Tablets** *Sufficient for 300mL of sample*	30 tablets
88661	**Pierce Protease Inhibitor Tablets, EDTA-Free** *Sufficient for 300mL of sample*	30 tablets
88662	**Pierce Phosphatase Inhibitor Tablets** *Sufficient for 200mL of sample*	20 tablets
88663	**Pierce Protease and Phosphatase Inhibitor Tablets** *Sufficient for 200mL of sample*	20 tablets
88664	**Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-Free** *Sufficient for 200mL of sample*	20 tablets



reduce sample viscosity
in bacterial extracts

The initial steps in most protein extraction processes are cell lysis followed by clarification of the lysate to remove cell debris and nucleic acids. These are two of the most critical steps in optimizing protein yield and improving downsteam purification processes. Nucleic acid endonucleases reduce the viscosity of cellular lysates by digesting both DNA and RNA, improving pellet formation, and increasing protein yield. Unlike DNase I and most other endonucleases, Pierce Universal Nuclease for Cell Lysis has high activity on both double- and single-stranded DNA and RNA, hydrolyzing these polymeric nucleic acids into small subunits (1-3 bases in length).

Thermo Scientific Pierce Universal Nuclease for Cell Lysis

Thermo Scientific Pierce Universal Nuclease for Cell Lysis is ideal for complete digestion of nucleic acids when preparing cell lysates.

Pierce Universal Nuclease for Cell Lysis is a genetically engineered endonuclease from *Serratia marcescens*. The endonuclease degrades single-stranded, double-stranded, linear and circular DNA and RNA and is effective over a wide range of temperatures and pH. The Pierce Universal Nuclease has high specific activity that is 100-fold greater than DNase I with increased thermal stability compared to other nucleases. The enzyme completely digests nucleic acids to oligonucleotides that are less than five bases long. Pierce Universal Nuclease is ≥99% pure enzyme and is free of any measurable protease activity. Pierce Universal Nuclease for Cell Lysis is comparable in performance to Benzonase® Nuclease. The addition of Pierce Universal Nuclease to bacterial lysis buffer, such as B-PER Reagent, results in improved lysis and protein purification (Figure 1).

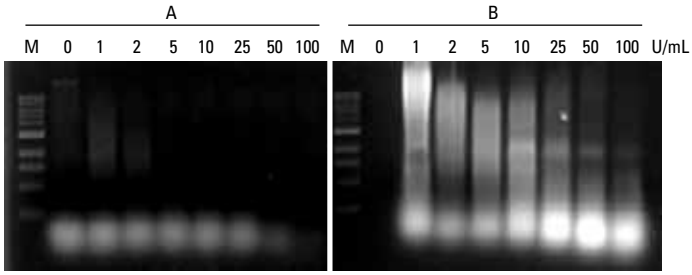


Figure 1. Thermo Scientific Pierce Universal Nuclease activity in cells lysed with Thermo Scientific B-PER Reagent. Cells were suspended in Thermo Scientific B-PER Reagent **(A)** or B-PER Reagent with lysozyme **(B)** with increasing concentrations of Pierce Universal Nuclease for Cell Lysis and incubated at room temperature for 30 minutes. The lysates were then cleared by centrifugation and resolved on a 1% agarose gel, and nucleic acids were stained with ethidium bromide and visualized under ultraviolet (UV) light. M, DNA ladder. Cells that were lysed with B-PER® Reagent with lysozyme but without Pierce Universal Nuclease for Cell Lysis were too viscous to be loaded onto the gel. Both images are from the same gel but were separated for presentation.

Highlights:

- **Broad spectrum** – degrades all forms of DNA and RNA
- **Highest-quality enzyme** – nuclease is ≥99% pure, as tested by SDS-PAGE
- **Robust activity** – 100-fold greater specific activity than DNase I
- **Versatile** – can be used with a wide variety of cell lysis reagents

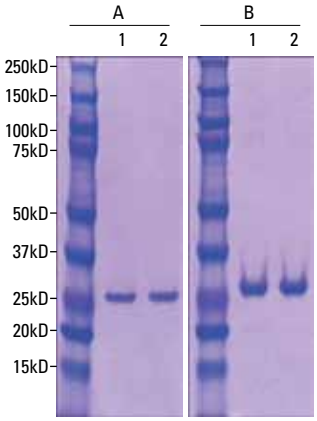


Figure 2. Thermo Scientific Pierce Universal Nuclease for Cell Lysis purity is comparable to that of other commercial nucleases. Pierce Universal Nuclease for Cell Lysis **(1)** and competitor E **(2)** were resolved by SDS-PAGE at a concentration of 3µg **(A)** or 8µg **(B)**. Band intensities were stained with Thermo Scientific Imperial Protein Stain.

Ordering Information

Product #	Description	Pkg. Size
88700	**Pierce Universal Nuclease for Cell Lysis** *Sufficient for 200mL lysate*	5kU
88701	**Pierce Universal Nuclease for Cell Lysis** *Sufficient for 1L lysate*	25kU
88702	**Pierce Universal Nuclease for Cell Lysis** *Sufficient for 4L lysate*	100kU

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