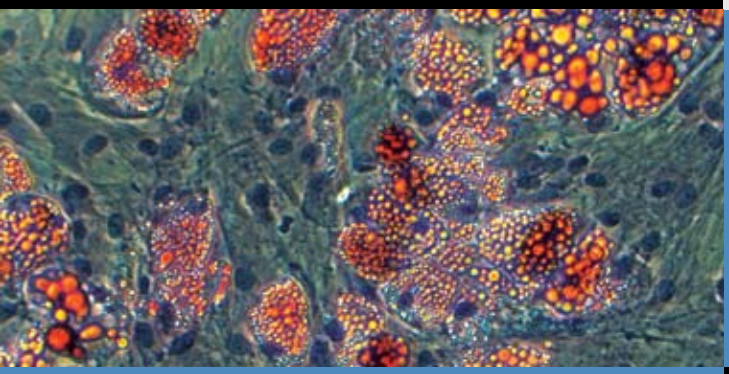
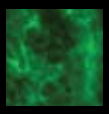


Thermo Scientific HyClone AdvanceSTEM™ Cell Culture Products

Advancing Stem Cell Research

AdvanceSTEM Cell Culture Products



ES Qualified Stem Cell Products

AdvanceSTEM Mesenchymal Maintenance

Thermo SCIENTIFIC

Thermo Scientific HyClone Products

Advancing Stem Cell Research

The number of studies involving stem cells has increased considerably over the past few decades, with a corresponding increase in the demand for sera and media designed specifically for stem cell applications. As stem cell research advances through clinical trials and into the therapeutic arena, we are uniquely positioned to serve your cell culture needs.

The Thermo Scientific HyClone Products are manufactured in Logan, Utah, a manufacturing facility capable of producing cell culture media and reagents using state-of-the-art manufacturing and filling equipment. These manufacturing systems have been validated for consistency and performance in liquid media lot sizes as large as 10,000 liters, and in powdered media lot sizes up to 6500kg.

For those concerned about the presence of animal-origin components, the Logan media and reagent manufacturing site has introduced a dedicated animal component free facility for the production of powdered media. In addition, we also feature HyNetics single use technology, which uses a biopharmaceutical-grade film in a closed environment to manufacture the purest, highest quality product.

Liquid media lots can be packaged in a number of configurations including standard bottles or customized BioProcess Container™ (BPC®) systems. These BPC systems enable simplified media handling for small-scale cell culture vessels or large-scale bioreactors and can be customized to provide specific volume sizes, from 1 to 1000 liters.

Typical customization of cell culture media and reagent formulations by our research and product development group includes nutrient demand, key component toxicities, necessary process specific supplementation, and other cell culture process optimization.

For a complete list of the Thermo Scientific HyClone AdvanceSTEM™ cell culture products, please visit our Web site at **www.thermo.com/hyclone/advancestem**. We look forward to working with you.

The Unique Properties of Stem Cells

Our cell culture experts understand the unique features of stem cells and their special needs. Stem cells are defined by two criteria common to all stem cells: their ability for prolonged undifferentiated proliferation, and their ability to differentiate into more than one type of specialized cell type. There are two broad categories of stem cells, pluripotent and multipotent. The most commonly studied pluripotent stem cell is the embryonic stem cell (ESC), which is derived from the inner cell mass of a blastocyst stage embryo. Multipotent stem cells or adult stem cells have been identified and isolated from most of the major tissues, including bone marrow derived mesenchymal stem cells.

ESCs are unique diploid cells that are capable of long-term proliferation in an undifferentiated state while maintaining the capacity for seemingly unlimited differentiation potential. The 1998 report of the derivation of human ESCs has sparked the imagination of scientists and laymen alike with the hope of learning how to control and direct the differentiation of these cells in order to study the earliest stages of human development, how specialized tissues and organs are generated, and how we might be able to use these cells to develop therapies that might be able to treat complex degenerative diseases that current therapies cannot adequately address.

Mouse ESCs have been used by biologists to study mammalian development and model human diseases for the past 25 years. In addition, they have become a standard research tool. ESCs are sensitive to culture conditions and can easily differentiate and lose pluripotency if not properly maintained. We set the standard for high-quality fetal bovine serum used for the culture of embryonic stem cells and provide lot tested batches that have been carefully analyzed for their ability to support the growth of mESCs. As part of continuing efforts to address the research and development needs for the highest-quality, stem cell culture reagents, we are building on our sera and adding new reagents that are carefully formulated to support the culture of pluripotent mESCs.

Multipotent stem cells, sometimes called adult stem cells (ASCs), have been found to persist in their undifferentiated state in embryonic and adult tissues. These cells can be derived from most tissues and, depending on their origin, have different properties and capacity for generating a variety of progeny. However, unlike embryonic stem cells, they are limited in quantity, have limited life span in culture and have a limited number of cell types into which they can differentiate. Mesenchymal stem cells represent an interesting and widely used stem cell type, possessing the ability to differentiate into adipocytes, chondrocytes and osteocytes.

The Thermo Scientific HyClone AdvanceSTEM line of stem cell culture products has been developed specifically for applications in stem cell research.

The line comprises numerous products designed to support a wide range of applications such as the expansion and maintenance of undifferentiated murine embryonic and human adult mesenchymal stem cells, to the directed differentiation of mesenchymal stem cells into adipocytes, chondrocytes, and osteocytes.

Thermo Scientific HyClone AdvanceSTEM™ Cell Culture Products

Providing optimal culture conditions can be one of the greatest challenges in stem cell research, particularly in keeping cells in an undifferentiated state or directing differentiation when desired.

The Thermo Scientific HyClone AdvanceSTEM™ line of stem cell culture products can help you meet those challenges.

Thermo Scientific HyClone AdvanceSTEM Serum Replacement

Our serum replacement has been developed to support the growth of undifferentiated embryonic stem cells in culture with no requirement for fetal bovine serum supplementation.

Ordering Information

Part Number	Thermo Scientific HyClone Product	Size
SH30874.02	AdvanceSTEM Serum Replacement	100mL
SH30874.03	AdvanceSTEM Serum Replacement	500mL

Thermo Scientific HyClone AdvanceSTEM Low Osmo DMEM

This basal medium has an optimized osmolality which approximates that of murine embryonic tissue. The formulation specifically supports the growth and maintenance of murine ESCs in an undifferentiated state.

Ordering Information

Part Number	Thermo Scientific HyClone Product	Size
SH30870.01	AdvanceSTEM Low Osmo DMEM Without L-Glutamine	500mL
SH30870. 02	AdvanceSTEM Low Osmo DMEM Without L-Glutamine	1000mL

Some murine embryonic stem cell lines have been observed to double more rapidly in AdvanceSTEM Low Osmo DMEM than classical DMEM formulations. Following good cell culture techniques, cells should be microscopically observed daily to monitor status.

ES Qualified Stem Cell Products

Classic culture conditions for murine embryonic stem cells (mESCs) include culturing in medium containing DMEM, FBS, LIF (Table 1), and co-culturing with primary mouse embryonic fibroblasts (MEFs). Pluripotent mESCs have a very distinct morphology when cultured under these conditions; growing as tightly clustered colonies with smooth phase bright borders. Mouse ESC's grow quickly and require daily maintenance.

Table 1: Preparation of 200mL B6 ES cell medium

Brand	Amount for 200mL	Product	Catalog Number
Thermo Scientific	145mL	HyClone AdvanceSTEM IMDM4SC	**SH30822**
Thermo Scientific	40mL	HyClone ES Screened FBS	**SH30070(E)**
Thermo Scientific	2.0mL	HyClone AdvanceSTEM ES Qualified L-glutamine 200mM	**SH30852**
Thermo Scientific	2.0mL	HyClone AdvanceSTEM ES Qualified Non-Essential Amino Acids (NEAA) 100X	**SH30853**
Thermo Scientific	2.0mL	Penicillin/Streptomycin Solution (optional)	**SV30010**
Thermo Scientific	2.0mL	HyClone AdvanceSTEM ES Qualified HEPES (1M)	**SH30851**
Fisher	3.0µL	2-ME	**ICN19470580**
Millipore	20µL	ESGRO LIF	**ESG1107**

Mix all ingredients in the top of a 250mL PES filter 0.22µm unit and filter sterilize. Store at 4°C. Discard unused medium after 10 days.

Pluripotent B6-ESC's have a very distinct morphology when cultured under certain conditions, growing as tightly clustered colonies with smooth phase bright borders. The PRX- B6 ESCs grow quickly and require daily maintenance. When co-cultured in the medium described in Table 2 with mouse embryonic fibroblasts (MEFs), these B6 ESCs are very robust.

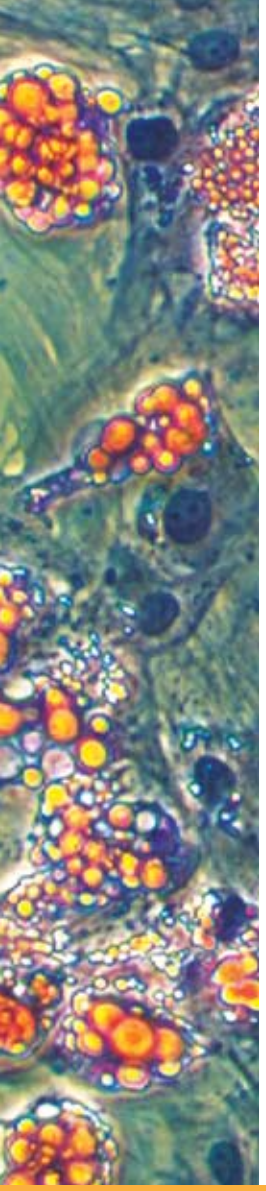
Table 2: MEF Medium (total volume = 500mL)

Brand	Amount for 500mL	HyClone Product	Catalog Number
Thermo Scientific	440mL	AdvanceSTEM DMEM4SC	**SH30824**
Thermo Scientific	50mL (10% of final volume)	ES Screened FBS	**SH30070(E)**
Thermo Scientific	5.0mL	AdvanceSTEM ES Qualified L-glutamine 200mM	**SH30852**
Thermo Scientific	5.0mL	AdvanceSTEM ES Qualified Non-Essential Amino Acids (NEAA) 100X	**SH30853**
Thermo Scientific	5.0mL	Penicillin/Streptomycin Solution (optional)	**SV30010**

Aseptically combine medium, FBS, and supplements, then mix by gently inverting a closed container. Store at 4°C. Unused medium should be discarded after six weeks.

Ordering Information

Part Number	Thermo Scientific HyClone Product	Size
SH30822.01	AdvanceSTEM IMDM4SC	500mL
SH30824.01	AdvanceSTEM DMEM4SC without L-Glutamine	500mL
SH30851.01	AdvanceSTEM ES Qualified HEPES	100mL
SH30852.01	AdvanceSTEM ES Qualified L-Glutamine	100mL
SH30853.01	AdvanceSTEM ES Qualified NEAA	100mL



Thermo Scientific HyClone Embryonic Stem (ES) Cell Screened Fetal Bovine Serum, U.S. Origin

For research involving embryonic stem cells (ESCs), it is critical to maintain them in their undifferentiated state. Historically, researchers were required to screen several lots of serum to find one suitable for ESC research. Thermo Scientific HyClone ES Screened FBS has been screened for the ability to promote the rapid growth of ES cells while retaining their pluripotent state, eliminating the need for customers to prescreen serum lots. This saves you both time and money, while providing the assurance that the serum used has been screened by experienced ESC culturists.

The screening includes plating efficiency, colony morphology, and toxicity tests. This protocol was adapted from *Gene Targeting*, A.L. Joyner, 1995, page 43. ES screened FBS is selected from our highest quality defined FBS lots, and it is filtered through sequential 40nm (0.04µm) pore-size rated filters. Further processing includes heat inactivation or irradiation, and can be done upon request.

Ordering Information

Part Number	Thermo Scientific HyClone Product	Size
SH30070.02E	HyClone ES Screened FBS, US Origin	100mL
SH30070.03E	HyClone ES Screened FBS, US Origin	500mL

Mesenchymal Maintenance with AdvanceSTEM™

Human Mesenchymal Stem Cells are primary cells which can be successfully cultured approximately eight passages. Thermo Fisher Scientific offers many options for the expansion of hMSCs, as well as directed adipogenic, osteogenic, and chondrogenic differentiation.

Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell Expansion Kit

This kit has been specifically formulated to support the optimal expansion and maintenance of undifferentiated hMSCs, and includes 500mL of AdvanceSTEM Mesenchymal Stem Cell Basal Medium and 50mL of AdvanceSTEM Growth Supplement.

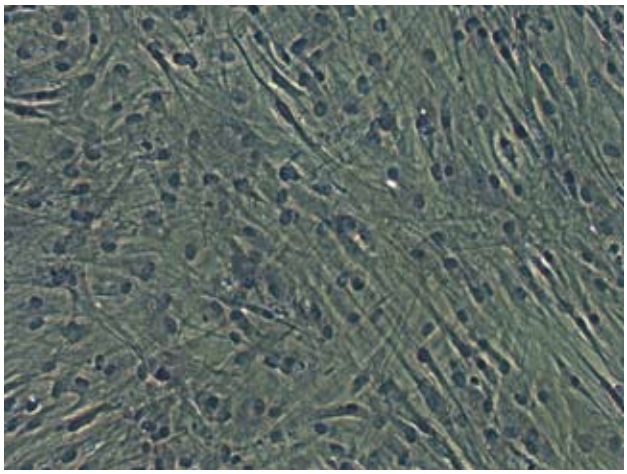
Ordering Information

Part Number	Thermo Scientific HyClone Product	Size
SH30875.KT	AdvanceSTEM Mesenchymal Stem Cell Expansion Kit	Kit Contains:
SH30878.01	AdvanceSTEM Stem Cell Growth Supplement	1 x 100mL
SH30879.02	AdvanceSTEM Mesenchymal Stem Cell Basal Medium	1 x 1000mL

Individual components are also available:

Ordering Information

Part Number	Thermo Scientific HyClone Product	Size
SH30878.01	AdvanceSTEM Stem Cell Growth Supplement	100mL
SH30879.01	AdvanceSTEM Mesenchymal Stem Cell Basal Medium	500mL
SH30879.02	AdvanceSTEM Mesenchymal Stem Cell Basal Medium	1000mL



Undifferentiated human mesenchymal stem cells stained with hematoxylin

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