

biopotential



Media and Matrices for Stem Cell Biology

Unlock Extraordinary Potential with
Media and Matrices from Sigma®

SIGMA-ALDRICH®

SIGMA® Where *bio* begins™
Life Science



Media

Optimize Your Stem Cell Expansion

Proven consistent results and optimized formulations have made Sigma's Stemline® Media a must-have for researchers rising to the challenges of adult stem cell expansion and maturation. Along with our broad selection of reagents, supplements, antibodies, and cytokines, Stemline Media ensures optimal expansion of robust cells.

The Stemline Media family includes

- Stemline Hematopoietic Stem Cell Expansion Medium I and II
- Stemline Neural Stem Cell Expansion Medium
- Stemline T cell Expansion Medium
- Stemline Dendritic Cell Maturation Medium
- Stemline Mesenchymal Stem Cell Expansion Medium
- Stemline Keratinocyte Medium II



Stemline® Hematopoietic Stem Cell Expansion Media

Features and Benefits

- Serum-free formulation
- Enhanced expansion from cord blood CD34+ cells
- Expands cells from all appropriate hematopoietic lineages in a colony-forming unit
- Tested extensively in 7-day and 14-day growth assays



Developed to promote the optimal expansion of human hematopoietic stem cells (HSC) from bone marrow, mobilized peripheral blood, and cord blood, Stemline Hematopoietic Stem Cell Expansion Medium demonstrates higher total nucleated cell (TNC) fold increases than other commercially available serum-free media formulations.

The second generation of Sigma's hematopoietic stem cell expansion media family, Stemline II, has been developed to optimize the balance of differentiated and undifferentiated cells while maximizing their expansion. Compatible with hematopoietic stem cells from bone marrow, cord blood, and mobilized peripheral blood, Stemline II has been shown to lead to significant increases in cell expansion from all three sources. Through flow cytometric analysis of clinical-scale expansions, Stemline II has also demonstrated higher capacity than other commercially available media for the expansion of CD34+/CD38+ late progenitors required for short-term engraftment. Human cord blood cells expanded in Stemline Media demonstrate impressive self-renewal when transplanted into immunodeficient NOC/SCID mice, illustrating Stemline's utility in a true functional trial.

Stemline Hematopoietic Stem Cell Expansion Medium is free of serum and all other animal-derived components with the exception of human serum albumin. This exclusion increases performance consistency and eliminates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Hematopoietic Stem Cell Expansion Medium is clearly an excellent choice for your HSC applications.

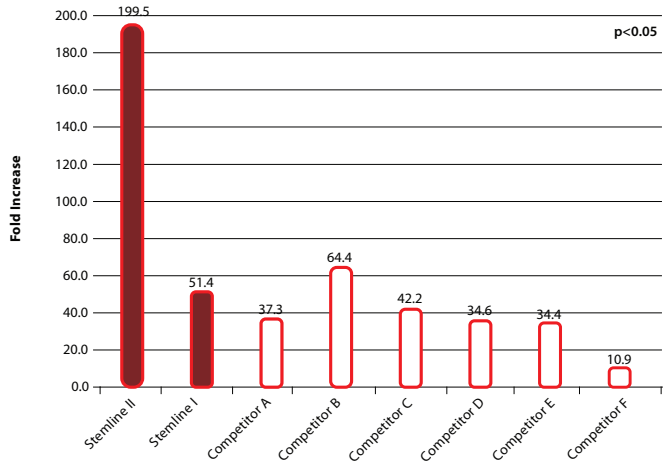


Figure 1. Fold Increase of Total Nucleated Cells from CD34+ Bone Marrow

Stemline demonstrates superior expansion of bone marrow hematopoietic stem cells (HSC). To test the ability of Stemline II Hematopoietic Stem Cell Expansion Medium to expand CD34+ HSCs, researchers at Sigma-Aldrich and the University of Kentucky designed a bench-scale expansion assay. Cells were seeded into the wells of 24-well tissue culture plates. One milliliter of medium was added to each well with the appropriate cytokines to stimulate growth (100 ng/mL each of TPO, SCF, and G-CSF). Each condition was performed in triplicate and seeded with 10,000 cells per ml in each well. Cells were counted on day 14 and the fold increase was determined by cells final/cells initial. HSCs from bone marrow cultured in Stemline and Stemline II demonstrated superior expansion to those grown in other serum-free HSC media.

Ordering Information

Cat. No.	Product Description	Size
50189	Stemline Hematopoietic Stem Cell Expansion Medium	500 mL
50192	Stemline II Hematopoietic Stem Cell Expansion Medium	500 mL

References:

1. Choong, M., *et al.*, MicroRNA expression profiling during human cord blood-derived CD34 cell erythropoiesis. *Experimental Hematology*, **35**, 551-564 (2007).
2. Levay, K., *et al.*, Tescalcin is an essential factor in megakaryocytic differentiation associated with Ets family gene expression. *Journal of Clinical Investigations*, **117**, 2672-2683 (2007).
3. Lu, S., *et al.*, Generation of functional human glioblasts from human embryonic stem cells. *Nature Methods*, **4**, 501-509 (2007).
4. McNiece, I., *et al.*, Delivering cellular therapies: lessons learned from ex vivo culture and clinical applications of hematopoietic cells. *Seminars in Cell & Developmental Biology*, **18**, 839-45 (2007).
5. Stec, M., *et al.*, Expansion and differentiation of CD14+CD16- and CD14++CD16+ human monocyte subsets from cord blood CD34+ hematopoietic progenitors. *Journal of Leukocyte Biology*, **82**, 594-602 (2007).
6. Wulf-Goldenberg, A., *et al.*, Cytokine pre-treatment of CD34+ cord blood stem cells *in vitro* reduces long-term cell engraftment in NOD/SCID mice. *European Journal of Cell Biology*, **87**, 69-80 (2007).

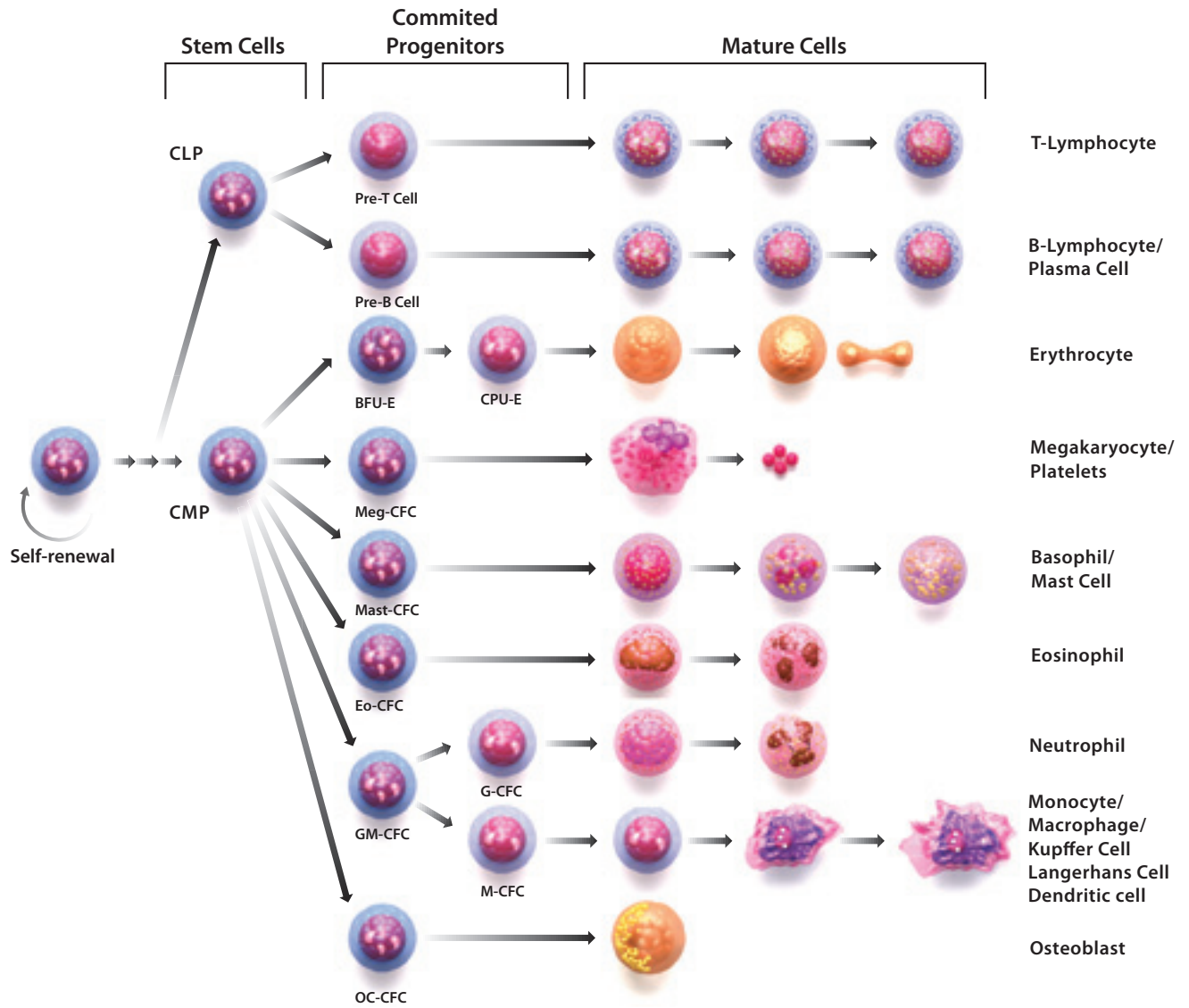


Figure 2. Hematopoietic Lineages
 Through the process of hematopoiesis, all cellular blood components are derived from hematopoietic stem cells.

Stemline® Neural Stem Cell Expansion Medium

Features and Benefits

- Serum-free formulation
- For use with neurosphere and monolayer cultures
- Cells retain differentiation capacity
- Superior expansion rates when compared to alternatives



Developed to promote optimal expansion of human neural stem cells (NSC), Stemline Neural Stem Cell Expansion Medium demonstrates rigorous expansion of human neural stem cells in both neurosphere and monolayer cultures.

Stemline Neural Stem Cell Expansion Medium is free of serum and all other animal components; this exclusion increases performance consistency and eliminates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Neural Stem Cell Expansion Medium is clearly an excellent choice for your NSC applications.

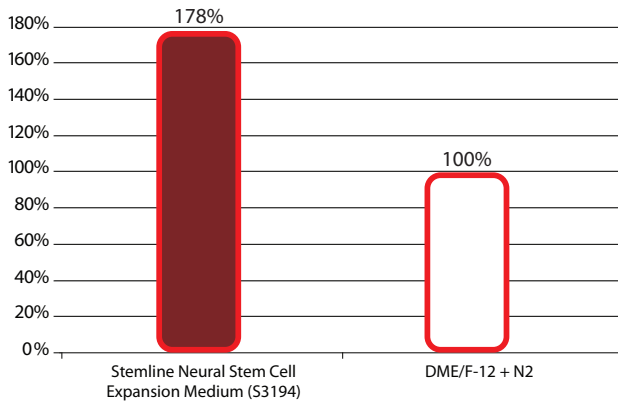


Figure 3. Growth of Neurospheres

Stemline demonstrates superior expansion of neural stem cell (NSC) neurospheres. To test the ability of Stemline Neural Stem Cell Expansion Medium to expand human NSC neurospheres, researchers at Sigma-Aldrich and the University of Wisconsin designed a bench-scale expansion assay. Cells were prepared using the method of Svendsen et al. Spheres were grown in standard DME/F-12 medium supplemented with 20 ng/mL EGF and 1% N-2 supplement prior to splitting. Half of the spheres remained in the N-2 supplemented medium and half were placed in Stemline Neural Stem Cell Expansion Medium (also supplemented with 20 ng/mL EGF). After several passages, overall proliferation was measured via BrdU incorporation. NSC neurospheres cultured in Stemline demonstrated superior expansion to those grown in other serum-free NSC media.

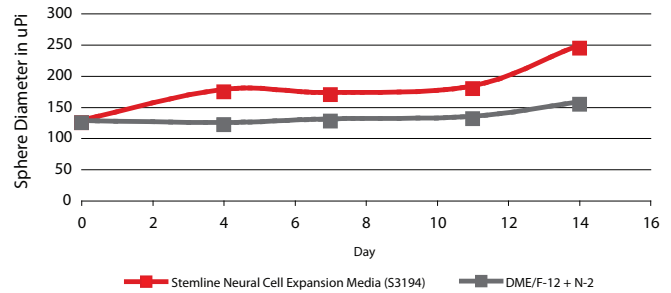


Figure 4. Growth of Neurospheres Expansion of Monolayer Neural Stem Cells in 24-well Culture Plate

Stemline demonstrates superior expansion of monolayer neural stem cells (NSC). To test the ability of Stemline Neural Stem Cell Expansion Medium to expand human monolayer NSCs, researchers at Sigma-Aldrich and the University of Wisconsin designed a bench-scale expansion assay. Cells were grown in monolayer format by seeding the cells at 20,000 cells/cm² on poly-L-lysine coated 24-well tissue culture plates. Cells were incubated for 5 days in medium supplemented with EGF (Cat. No. E9644) and LIF (Cat. No. L5283). After several passages, overall proliferation was measured. Monolayer NSC cultured in Stemline demonstrated superior expansion to those grown in other serum-free NSC media.

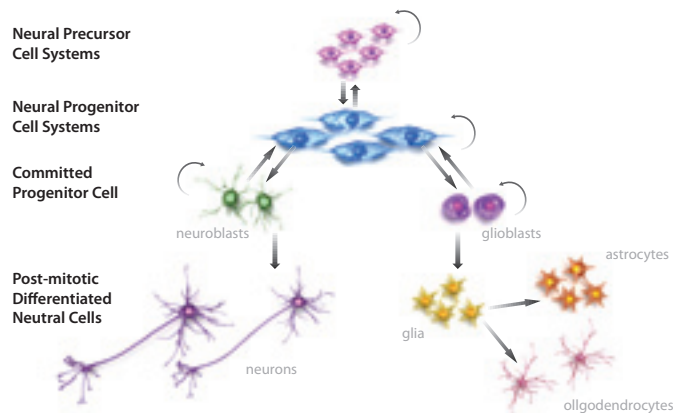


Figure 5. Growth of Neurospheres Neurogenesis

Ordering Information

Cat. No.	Product Description	Size
S3194	Stemline Neural Stem Cell Expansion Medium	500 mL

Stemline® T Cell Expansion Medium

Features and Benefits

- Serum-free formulation
- Excellent expansion of T cells of human origin
- Supports high cell densities that exhibit rigorous and consistent growth kinetics
- Maintains the proper CD4/CD8 ratio in flow cytometric analysis
- Maintains functionality, both *ex vivo* and *in vivo*



Developed to promote the optimal expansion of adult human T cells, Stemline T cell Expansion Medium demonstrates significantly greater expansion (55%) when compared to alternative media, and viability greater than 95%. Additionally, flow cytometry confirms that with Stemline, a proper CD4/CD8 ratio is maintained. In an *ex vivo* functional assay (⁵¹Chromium Release Assay), T cells expanded in Stemline medium proved to be highly functional and possessed cytolytic potential greater than T cells expanded in serum-containing alternative medium (RPMI with 10% fetal bovine serum). In an *in vivo* functional assay (GvHD Induction), human T lymphocytes expanded in Stemline medium were injected into NOD/SCIDβ2M mice (n=12). Engraftment, perivascular infiltration, and lethal GvHD were observed by day 15 in 100% of mice, demonstrating excellent *in vivo* expansion and functionality.

Stemline T cell Expansion Medium is free of serum and all other animal-derived components with the exception of human serum albumin, cholesterol, and transferrin. This exclusion increases performance consistency and eliminates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline T cell Expansion Medium is clearly an excellent choice for your T cell applications.

Reference:

1. Nervi, B., *et al.*, Factors affecting human T cell engraftment, trafficking, and associated xenogenic graft-vs-host disease in NOD/SCID β2mnull mice. *Experimental Hematology*, **35**, 1823-1838.

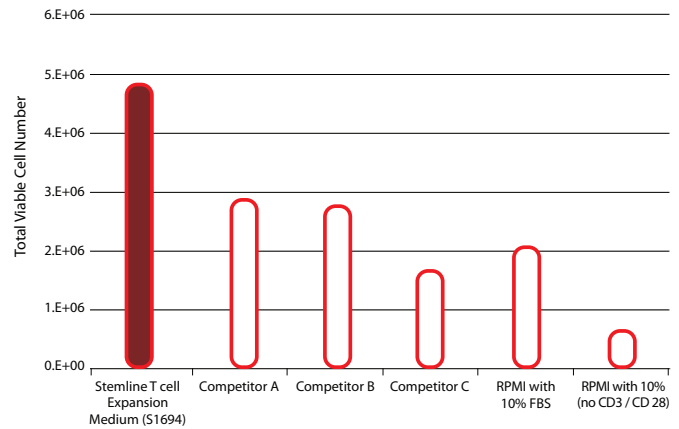


Figure 6. Growth of Neurospheres T Cell Expansion Day 7

Stemline demonstrates superior expansion of T cells. When compared with three alternative commercial media and two RPMI formulations, Stemline demonstrated >40% more total viable cells.

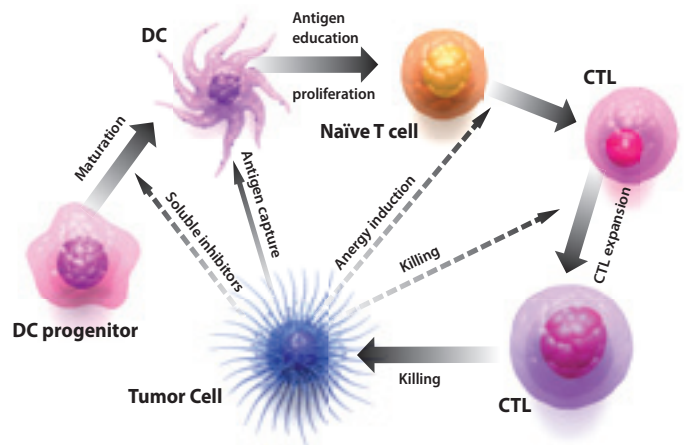


Figure 7. In-vitro Interaction of Dendritic Cells, T-Cells, and Tumor Cells

As Dendritic Cells begin to process antigens, they mature and exhibit a more star-shaped appearance. Mature Dendritic Cells process antigen and present it to Cytotoxic T cells. Activated Cytotoxic T cells now recognize the tumor and destroy it.

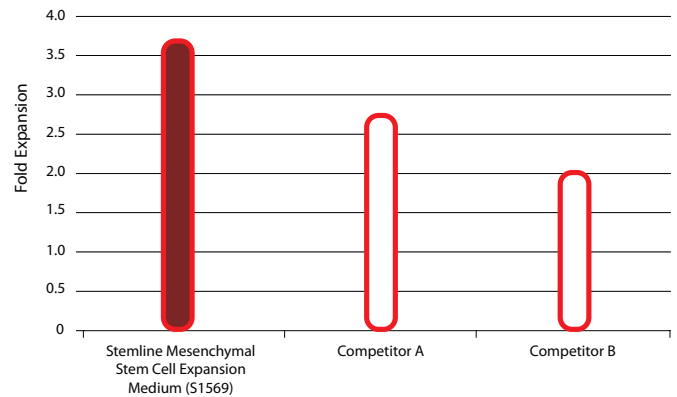
Ordering Information

Cat. No.	Product Description	Size
S1694	Stemline T-Cell Expansion Medium	500 mL

Stemline® Mesenchymal Stem Cell Expansion Medium

Features and Benefits

- Maximum expansion of CD34+ progenitors
- Supports robust, high density cell populations
- Superior expansion
- Cells retain their differentiation potential at 14 days in culture



Developed to promote optimal expansion of human mesenchymal stem cells (MSC) from bone marrow, Stemline Mesenchymal Stem Cell Expansion Medium demonstrates greater total nucleated cell (TNC) fold increases than other commercially available formulations. Additionally, functional trials clearly demonstrate Stemline's capacity to promote differentiation into adipocytes, chondrocytes, and osteocytes.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Mesenchymal Stem Cell Expansion Medium is clearly an excellent choice for your MSC applications.

Stemline Mesenchymal Stem Cell Expansion Medium requires supplementation with antibiotics, cytokines, L-glutamine and fetal bovine serum, as appropriate to individual research protocols. Known to be extremely sensitive during initial isolation and growth *ex vivo*, MSC proliferation depends highly on the composition of fetal bovine serum (FBS) used to supplement the medium. Pre-screening with FBS is recommended, as the specific FBS components that affect MSC growth have not been fully identified.

Figure 8. Stemline Demonstrates Superior Expansion of Mesenchymal Stem Cells (MSC)

To test the ability of Stemline Mesenchymal Stem Cell Expansion Medium to promote expansion of MSCs, researchers at Sigma-Aldrich designed a bench-scale assay. Triplicate 2 mL cultures at 5,000 MSCs/cm² were grown in a 6-well microplate culture system in Stemline medium or other media containing FBS. Each well was treated with trypsin/EDTA, triturated, and harvested after a 14-day expansion. MSCs were counted using a hemacytometer and average viable cell count determined for each condition. MSCs cultured in Stemline demonstrated superior expansion to those grown in other MSC media, retained their differentiation potential and were easily passaged routinely.

Ordering Information

Cat. No.	Product Description	Size
S1569	Stemline Mesenchymal Stem Cell Expansion Medium	1 L

Stemline® Keratinocyte Medium II

Features and Benefits

- Serum-free basal formulation
- Two supplement cocktails
- Regional expansion of NHEK cells



Developed to promote optimal expansion of human epidermal keratinocytes from adult and neonatal sources, Stemline Keratinocyte Medium II performs most effectively when supplemented with either Stemline Keratinocyte Growth Supplement (**Cat. No. S9945**) or Keratinocyte Medium Supplement, (**Cat. No. K3136**).

Stemline Keratinocyte Medium II is free of serum and all other animal components; this exclusion increases performance consistency and eliminates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility, Stemline Keratinocyte Stem Cell Expansion Medium is clearly an excellent choice for your keratinocyte applications.

Ordering Information		
Cat. No.	Product Description	Size
S0196	Stemline Keratinocyte Medium II	500 mL
S9945	Stemline Keratinocyte Growth Supplement	1 vial

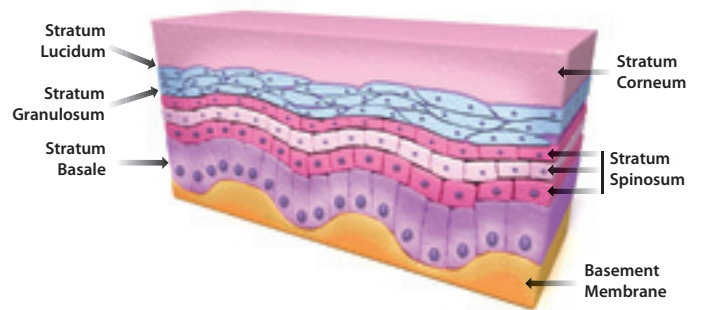


Figure 9. Migration of Keratinocytes

The epidermis is composed of 4 layers of keratinocytes. The stratum basale, the deepest layer, is composed of column-shaped cells that constantly divide and force existing cells into higher layers. As the cells migrate through these layers, they flatten and eventually undergo terminal differentiation, which leads to programmed cell death. The top layer, the stratum corneum, is composed of these dead keratinocytes, which are continuously rubbed off and replaced anew.

Stemline® Media Selection Guide

Name	Key Attributes	Animal Components	Required L-Glutamine Supplementation	Regulatory	Cat. No.	Size
Stemline I & II Hematopoietic Stem Cell Expansion Medium	Serum-free formulation	Human serum albumin	S0189 need L-Glutamine	Manufactured cGMP; DMF on file	S0189	500 mL
	Enhanced expansion from cord blood CD34+ cells				S0192	500 mL
	Expands cells from all appropriate hematopoietic lineages in a colony-forming unit					
	Tested extensively in development in 7-day and 14-day growth assays					
	For more information, see page 3-4					
Stemline Neural Stem Cell Expansion Medium	Serum-free formulation	None	None	Manufactured cGMP; DMF on file	S3194	500 mL
	For use with both neurosphere and monolayer cultures					
	Cells retain differentiation capacity					
	Superior expansion rates					
	For more information, see page 5					
Stemline T cell Expansion Medium	Serum-free formulation	Human serum albumin, cholesterol, Human transferrin	4 mM	Manufactured cGMP; DMF on file	S1694	500 mL
	Excellent expansion of T cells of human origin					
	Supports high cell densities that exhibit rigorous and consistent growth kinetics					
	Maintains the proper CD4/CD8 ratio in flow cytometric analysis					
	Maintains functionality, both <i>ex vivo</i> and <i>in vivo</i>					
	For more information, see page 6					
Stemline Dendritic Cell Maturation Medium	Serum-free formulation	Human serum albumin, cholesterol, Human transferrin	2 mM	Manufactured cGMP; DMF on file	S3444	1 L
	Supports high density cultures of mature dendritic cells					
	Cultures maintain morphological and phenotypic characteristics					
	Promotes maturation of DCs from human CD14+ monocytes					
	For more information, see page 7					
Stemline Mesenchymal Stem Cell Expansion Medium	Maximum expansion of CD34+ progenitors	Human transferrin; requires FBS supplementation	4 mM	Manufactured cGMP; DMF on file	S1569	1 L
	Supports robust, high density cell populations					
	Superior expansion					
	Cells retain their differentiation potential at 14 days in culture					
	For more information, see page 8					
Stemline Keratinocyte Medium II	Serum-free basal formulation	Requires supplementation with either S9945 or K3136	4 mM	Manufactured cGMP	S0196	500 mL
	Two supplement cocktails					
	Regional expansion of NHEK cells					
	For more information, see page 9					



Matrices

HyStem™ Cell Culture Scaffolds

The First Customizable Synthetic ECM

Sigma® is pleased to introduce HyStem, the first customizable synthetic ECM that closely mimics *in vivo* conditions, to enable three-dimensional culture of stem cells.

Customizable:

The HyStem platform offers you, the researcher, control over growth factor incorporation, attachment factor incorporation, ECM protein incorporation, rigidity of the hydrogel, and cell encapsulation vs. top plating.

Synthetic:

Because HyStem is a synthesized matrix and not a biological extract, researchers are able to closely control the composition of their cells' environment. HyStem's components include chemically synthesized HyStem (thioated hyaluronic acid), Extralink™ (thio-reactive cross-linker), degassed water, and biologically purified Gelin-S™ (denatured collagen).

Biologically Accurate:

HyStem kits are optimal for culturing stem cells whose natural environments are rich in hyaluronic acid. The HyStem hydrogel scaffold closely mimics the rich, natural extracellular matrix environment, complete with hyaluronic acid and collagen fibrils, while offering the flexibility to customize with appropriate growth factors, attachment factors, and proteins.

The HyStem platform includes three unique members:



HyStem

Cell Culture Scaffold Kit: For researchers who require an animal component-free system, for researchers who will customize with their own attachment factors and/or ECM proteins/peptides, and for researchers who require a minimal number of cell attachment sites.



HyStem-C

Cell Culture Scaffold Kit: For researchers who require a large number of generalized cell attachment sites for their stem cell culture(s).



HyStem-HP

Cell Culture Scaffold Kit: For researchers planning to incorporate and gradually release growth factors into the stem cell environment.

Natural Extracellular Environment

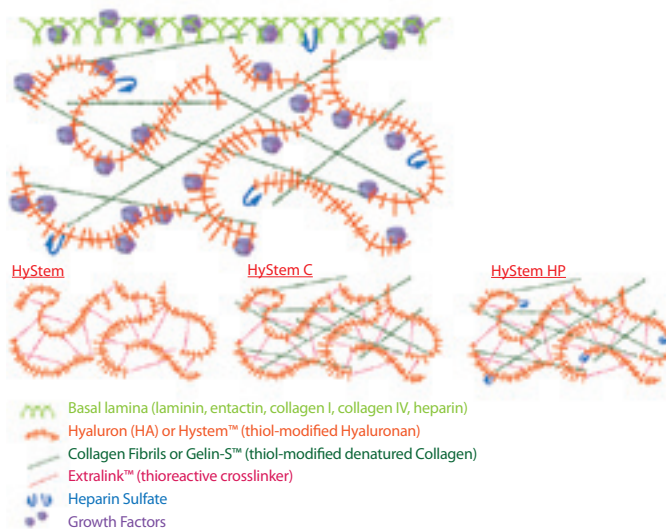


Figure 10. HyStem closely mimics the natural extracellular environment

Ordering Information

Cat. No.	Product Description	Volume of Hydrogel Produced
HYS010	HyStem Cell Culture Scaffold Trial Kit	2.5 mL
HYSC010	HyStem-C Cell Culture Scaffold Trial Kit	2.5 mL
HYSHPO10	HyStem-HP Cell Culture Scaffold Trial Kit	2.5 mL
HYS020	HyStem Cell Culture Scaffold Kit	7.5 mL
HYSC020	HyStem-C Cell Culture Scaffold Kit	7.5 mL
HYSHPO20	HyStem-HP Cell Culture Scaffold Kit	7.5 mL
H2666	HyStem-C 96-well plate	96 wells

HyStem Closely Mimics the Natural Extracellular Environment

Application Data

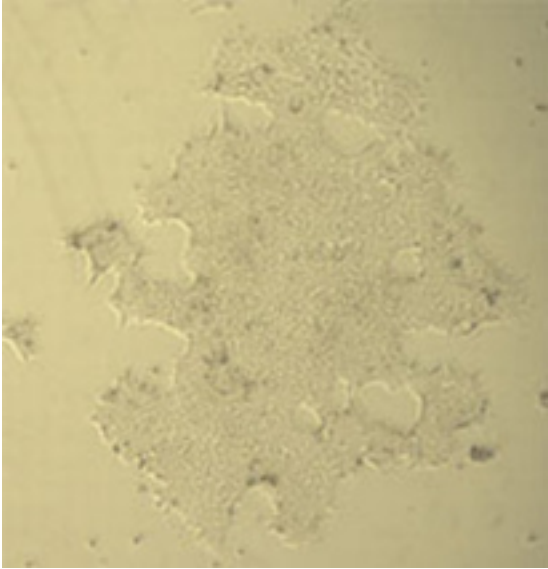


Figure 11. H9 human embryonic stem cells plated on HyStem hydrogels containing CVFL and grown for 3 days.

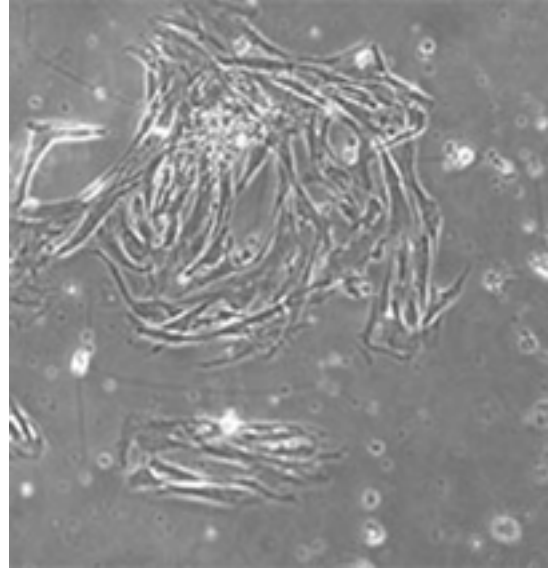


Figure 12. Human mesenchymal stem cells grown (5 days) on the surface of a HyStem hydrogel with collagen I non-covalently incorporated.

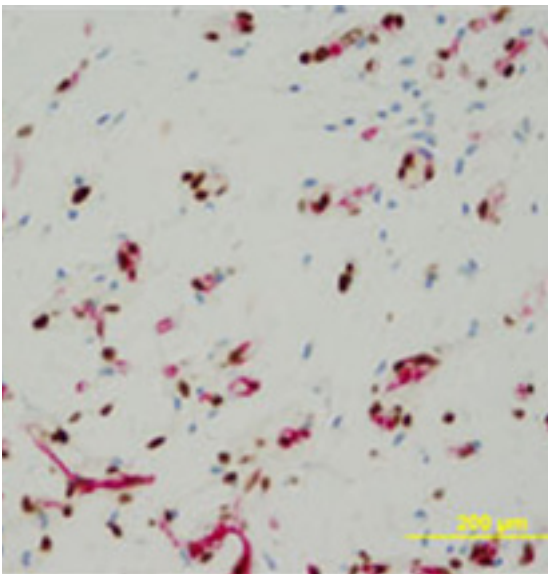


Figure 13. Endothelial progenitor cells cultivated in HyStem-HP. Blue = mouse cell nuclei, brown = human cell nuclei, red = CD31 protein (courtesy of Robert Grove, Endgenitor Technologies, Inc., Indianapolis, IN)

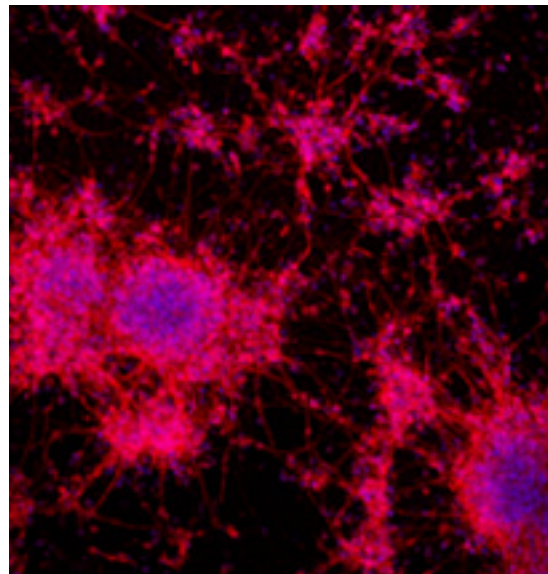


Figure 14. Neurosphere-derived human embryonic stem cells (H9) seeded in HyStem-C and grown for 5 days. Red = β -Tubulin III. Blue = Draq-5.

To learn more, visit
sigma.com/hystem

HyStem™ Cell Culture Scaffold Kit Protocol and Technical Information

HyStem is recommended for applications requiring attachment factor optimization. Extracellular matrix proteins can be mixed into the hydrogel and incorporated non-covalently before gelation. Alternatively, attachment peptides having an N-terminal cysteine can also be covalently linked to the matrix.³

HyStem Hydrogel Kits are optimal for culturing stem cells whose natural environment is rich in hyaluronic acid (HA). It is xeno-free since its two components are thiol-modified hyaluronan (HyStem) and a thiol-reactive crosslinker (polyethylene glycol diacrylate, Extralink™). HyStem can be customized by adding extracellular matrix (ECM) proteins² or cell attachment peptides³ into the hydrogel to provide attachment site and/or differentiation signals. They can also be varied by changing the hydrogel rigidity⁴ to match that of the native cell environment.

Animal-Free HyStem

Hyaluronic acid used to produce HyStem is made by a proprietary bacterial-fermentation process using *Bacillus subtilis* (Novozymes). It is 100% free of animal-derived raw materials and no animal-derived ingredients are used in its production. Extralink (polyethylene glycol diacrylate) is made by adding acrylate groups to both ends of a polyethylene glycol (PEG) polymer. PEG is derived from petroleum and inorganic sources, and contains no animal source materials.

Gelation

Reconstituted HyStem components are liquids at 15–37 °C. The hydrogel is formed when the crosslinking agent, Extralink, is added to the HyStem liquid. Once the two components are mixed, gelation occurs in less than 20 minutes. There are no low temperature or low pH steps. The gelation time can be increased by diluting the components with phosphate buffered saline (PBS) or cell culture medium.

Flexibility

HyStem gives the researcher complete control over:

- Hydrogel rigidity
- Amount and type of ECM protein incorporated
- Cell attachment peptide incorporation
- Cell encapsulation or plating on top of hydrogel
- Cell growth format (96 to 6–well plates and/or tissue culture inserts)

Applications

Stem cell expansion

HyStem provides a basic, viscoelastic matrix for stem cell growth. This matrix can be manipulated by the user by changing its:

- Composition
- Rigidity

Procedure

The 1× Stock Solutions remain liquid at 15–37 °C. The hydrogel is formed when the crosslinking agent, Extralink, is added to the HyStem (thiol-modified hyaluronan). Gelation occurs in ~20 minutes after the solutions are mixed. No steps depend on low temperature or low pH.

The rigidity of the hydrogel can be varied either by changing the volume of 1× Extralink 1 Stock Solution used for crosslinking or by diluting the 1× HyStem Stock Solution using PBS or cell culture medium. Diluting the 1× HyStem Stock Solution with PBS or cell culture medium can increase the gelation time. The standard HyStem hydrogel results in a rigidity of ~300 Pa.

The following is a procedure to prepare a 2.5 mL batch of hydrogel scaffold. Sufficient reagents are provided to prepare 3 batches (7.5 mL).

1. Allow the HyStem (2 bottles), Extralink 1 (1 bottle), and degassed water bottles to come to room temperature.
2. Under aseptic conditions, using a syringe and needle, add 1.0 mL of degassed water (**Cat. No. W3894**) to each HyStem bottle.
3. Place the bottles horizontally on a rocker or shaker. It will take <30 minutes for the solids to fully dissolve. Warming to 37 °C and/or gently vortexing will speed dissolution. The 1× Stock Solutions will be clear and slightly viscous.
4. Under aseptic conditions, using a syringe and needle, add 0.5 mL of degassed water (**Cat. No. W3894**) to the Extralink 1 bottle. Invert several times to dissolve.
5. Mix the 2 bottles of 1× HyStem Stock Solution together. To mix, pipette back and forth slowly to avoid trapping air bubbles.
6. If adding ECM proteins, add sterile ECM protein solution to the HyStem 1× Stock Solution. Pipette back and forth to mix.
7. If encapsulating cells, resuspend the cell pellet in the HyStem 1× Stock Solution. Pipette back and forth to mix.
8. To form the hydrogel, combine the following and mix by pipette:
 - 0.5 mL of 1× Extralink 1 Stock Solution
 - 2.0 mL of the 1× HyStem Stock Solution.
9. Gelation will occur within <20 minutes.

Ordering Information

Cat. No.	Product Description	Volume of Hydrogel Produced
HYS010	HyStem Cell Culture Scaffold Trial Kit	2.5 mL
HYS020	HyStem Cell Culture Scaffold Kit	7.5 mL

HyStem™-C Hydrogel Kit

HyStem-C is recommended for stem cell researchers as a starting point for optimization of a cell's microenvironment since it contains Gelin-S™ or thiolated gelatin (denatured collagen), which allows attachment of a wide variety of cell types and takes the guesswork out of the appropriate attachment factors to use.

HyStem-C provides an excellent starting point for optimizing the matrix for stem cell culture since its composition can be easily tailored to find suitable matrix compositions. Unlike an animal-derived extracellular matrix (ECM), HyStem-C is fully chemically defined. The hydrogels are based on three biocompatible components: thiol-modified hyaluronan (a major constituent of native ECM), thiol-modified gelatin (denatured collagen), and a thiol-reactive crosslinker, polyethylene glycol diacrylate (PEGDA). HyStem-C hydrogels can be customized by adding ECM proteins and by varying the hydrogel rigidity to match the stiffness of native tissues.

Gelation

Reconstituted HyStem-C components remain liquid at 15–37 °C. The hydrogel is formed when the crosslinking agent, Extralink™ (PEGDA) is added to a mixture of HyStem (thiol-modified hyaluronan) and Gelin-S (thiol-modified gelatin). Gelation occurs in about twenty minutes after all three components are mixed. No steps depend on low temperatures or low pH. Diluting the components with phosphate-buffered saline (PBS) or cell-culture medium can increase the gelation time.

Flexibility

HyStem-C allows customization of experiments:

- Dimensionality (3-D encapsulation or 2-D plating on top of hydrogel)
- Cell-growth format (96 to 6-well plates and/or tissue-culture inserts)
- Amount and type of ECM protein incorporated
- Hydrogel rigidity

Applications

3-D Stem Cell Culture

In addition to stem cell culture on top of the hydrogel, HyStem-C provides the basic scaffold for 3-D stem cell growth. Stem cells can be encapsulated during crosslinking,¹ where they attach and grow within the hydrogel matrix, or they can be plated on top of the hydrogel for pseudo 3-D growth.² Cells are recovered from the hydrogel either by enzyme digestion for cells encapsulated in the hydrogel^{2,3} or by trypsinization for cells grown on the surface.

Gelin-S provides basic cell-attachment sites for cell lines and primary cells.^{2,3} Several cell types depend on specific ECM components, such as the natural ECM proteins laminin, collagen, fibronectin, and vitronectin, to grow and differentiate—all of which may be added to the HyStem-C hydrogel. These proteins are easily incorporated noncovalently into the hydrogel prior to gel formation. We recommend HyStem for incorporating ECMs.

Rigidity of the HyStem-C hydrogel can be varied either by changing the amount of Extralink used for cross-linking⁴ or by diluting the HyStem and Gelin-S solutions using PBS or cell-culture medium.

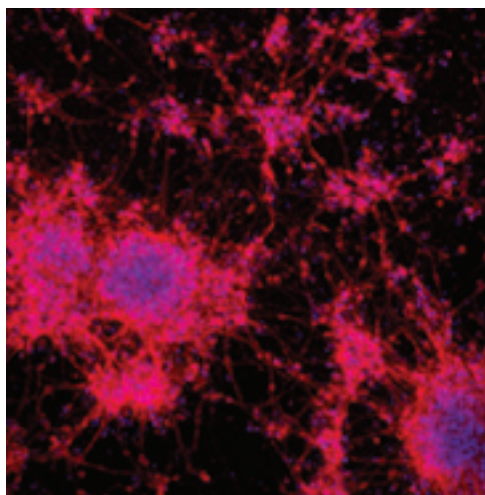


Figure 15. Neurosphere-derived human embryonic stem cells (H9) seeded in HyStem + Gelin-S hydrogels and grown for 5 days. Red = Beta III Tubulin. Blue = Draq-5.

A variety of stem cells (human embryonic, human mesenchymal stem cells⁴) and progenitor cells (neural progenitor, hepatic progenitor) have been cultured in HyStem-C.

Choosing a HyStem Hydrogel Kit

The HyStem-C Hydrogel Kit is designed to make hydrogels with 50 wt% HyStem and 50 wt% Gelin-S and is optimal for researchers who need a large number of generalized cell attachment signals for their cultures. The HyStem Hydrogel Kit is appropriate for researchers who will either add ECM proteins or who require a minimal number of cell attachment sites. If growth factors will be used, the HyStem-HP hydrogel kit is recommended. For *in vivo* experimentation, we recommend either the HyStem or HyStem-HP Hydrogel Kits.

Ordering Information		
Cat. No.	Product Description	Volume of Hydrogel Produced
HYSC010	HyStem-C Cell Culture Scaffold Trial Kit	2.5 mL
HYSC020	HyStem-C Cell Culture Scaffold Kit	7.5 mL
H2666	HyStem-C 96-well plate	96 wells

Procedure

Rigidity of the hydrogel can be varied either by changing the volume of 1× Extralink 2 Stock Solution used for crosslinking⁴ or by diluting the 1× HyStem and Gelin-S Stock Solutions using PBS or cell culture medium. Diluting these Stock Solutions with PBS or cell culture medium can increase the gelation time.

The following is a procedure to prepare a 2.5 mL batch of hydrogel scaffold.

1. Allow the HyStem, Gelin-S, Extralink 2, and degassed water bottles to come to room temperature.
2. Under aseptic conditions, using a syringe and needle, add 1.0 mL of degassed water (**Cat. No. W3894**) to the HyStem bottle. Repeat for the Gelin-S bottle.
3. Place both bottles horizontally on a rocker or shaker. It will take <30 minutes for the solids to fully dissolve. Warming to 37 °C and/or gently vortexing will speed dissolution. 1× Stock Solutions will be clear and slightly viscous.
4. Under aseptic conditions, using a syringe and needle, add 0.5 mL of degassed water (**Cat. No. W3894**) to the Extralink 2 bottle. Invert several times to dissolve.
5. As soon as possible, but within 2 hours of making the solutions, aseptically mix the HyStem and Gelin-S 1× Stock Solutions together. To mix, pipette back and forth slowly to avoid trapping air bubbles.
6. If adding other ECM proteins, add sterile ECM protein solution to the 1:1 mixture of HyStem and Gelin-S 1× Stock Solutions. Pipette back and forth to mix.
7. If encapsulating cells, resuspend the cell pellet in the 1:1 mixture of HyStem and Gelin-S 1× Stock Solutions. Pipette back and forth to mix.
8. To form the hydrogel, combine the following and mix by pipette: 0.5 mL of 1× Extralink 2 Stock Solution 2.0 mL of HyStem/Gelin-S 1:1 mixture
9. Gelation will occur within ~20 minutes.

References

1. G. D. Prestwich, Y. Liu, M. Serban, B. Yu, X. Z. Shu, and A. Scott, "3-D Culture in Synthetic Extracellular Matrices: New Tissue Models for Drug Toxicology and Cancer Drug Discovery," invited, *Adv. Enz. Res.*, in press (2007).
2. X. Z. Shu, S. Ahmad, Y. Liu, and G. D. Prestwich, "Synthesis and Evaluation of Injectable, In Situ Crosslinkable Synthetic Extracellular Matrices (sECMs) for Tissue Engineering," *J. Biomed Mater. Res. A*, **79A**(4), 901-912 (2006).
3. X. Z. Shu, Y. Liu, F. Palumbo, G. D. Prestwich, "Disulfide-crosslinked Hyaluronan-Gelatin Hydrogel Films: A Covalent Mimic of the Extracellular Matrix for *In Vitro* Cell Growth," *Biomaterials*, **24**, 3825-3834 (2003).
4. Unpublished data from Yongzhi Qiu, Robert McCall, Vladimir Mironov, Xuejun Wen, Clemson University, and Medical University of South Carolina.

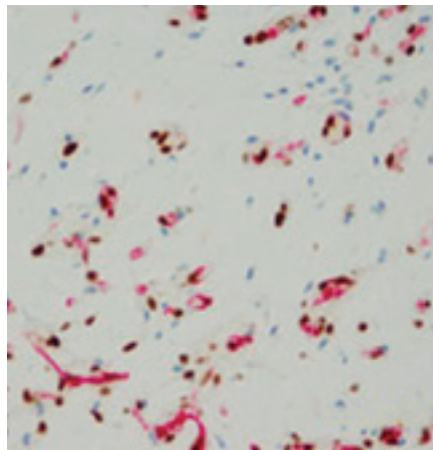


Figure 16. Endothelial progenitor cells cultivated in HyStem-HP. Blue = mouse cell nuclei, brown = human cell nuclei, red = CD31 protein (courtesy of Robert Grove, Endgenitor Technologies, Inc., Indianapolis, IN)

HyStem™-HP Hydrogel Kit

HyStem-HP is recommended for stem cell researchers whose applications require slow release of growth factors in a cell's microenvironment. HyStem-HP contains small amounts of thiolated heparin which ionically binds a wide variety of growth factors and slowly releases them over time.¹⁻³

HyStem-HP is ideal for stem cell applications where slowly released growth factors are crucial in recreating a stem cell niche. HyStem-HP hydrogels contains thiol-modified heparin which allows the slow release of growth factors (GFs) within an easily customizable, environment. HyStem-HP is a synthetic extracellular matrix (ECM) that can be injected and crosslinked *in situ*. Unlike an animal-derived ECM, HyStem-HP is chemically defined and nonimmunogenic. The HyStem-HP Hydrogel Kit contains HyStem-HP (a combination of thiol-modified hyaluronan, HA, and thiol-modified heparin), Gelin-S™ (thiol-modified gelatin), and Extralink™ (a thiol-reactive crosslinker, polyethylene glycol diacrylate, PEGDA).

Immobilized heparin in the hydrogel mimics the heparan sulfate proteoglycans normally present in the ECM. It also helps protect GFs from proteolysis and slows their release to attached cells.¹ This reduces the amount of GF required to achieve stimulation of cell growth or differentiation when compared to the use of free GF in media. All GFs tested to date (bFGF, VEGF, Ang-1, PDGF, TGFβ1, KGF) are released at different rates, but over a period of several weeks.¹⁻³

Gelation

Reconstituted HyStem-HP components remain liquid at 15–37 °C. The hydrogel is formed when Extralink is mixed with HyStem-HP and Gelin-S. Gelation occurs about twenty minutes after all three components are mixed. No steps depend on low temperature or low pH. Diluting the components with phosphate-buffered saline (PBS) or cell-culture medium can increase gelation time.

Flexibility

HyStem-HP ensures complete control over:

- Amount and type of GF incorporated
- Cell encapsulation or plating on top of hydrogel
- Cell-growth format (96 to 6-well plates and/or tissue-culture inserts)
- Amount and type of ECM proteins incorporated
- Hydrogel stiffness

Applications

3-D Stem Cell Growth

In addition to stem cell culture on top of the hydrogel, HyStem-HP provides the basic scaffold for 3-D stem cell growth. Cells can be encapsulated during crosslinking,⁴ where they attach and grow within the hydrogel matrix, or they can be plated on top of the hydrogel for pseudo 3-D growth.⁵ Cells are recovered from the hydrogel either by enzyme digestion for cells encapsulated in the hydrogel⁵ or by trypsinization for cells grown on the surface.

Gelin-S provides basic cell-attachment sites for stem cell lines. Several stem cell types depend on specific ECM components to grow and differentiate. To affect specific cell performance, other factors such as growth factors or ECM proteins may be added to the HyStem-HP hydrogel. ECM proteins are easily incorporated noncovalently into the hydrogel prior to gel formation as are growth factors.

3-D Stem Cell Growth Using GF-Supplemented Medium

For stem cells cultured with GFs in the medium, GFs may be removed from the medium and added to the HyStem-HP hydrogel. The hydrogel is used to coat a culture flask and cells are cultured on top of the hydrogel using medium without GFs. Note, however, that the GFs are released at different rates. Therefore, we recommend an *in vitro* test to determine the proper concentrations for GF addition to the hydrogel. See growth-factor release for more information about specific GFs and their retention.

Choosing a HyStem™ Hydrogel Kit

The HyStem™-HP Hydrogel Kit is designed to make hydrogels with 50 wt% HyStem-HP and 50 wt% Gelin-S™. If no GFs will be used, then we recommend the HyStem hydrogel kit.

References

1. S. Cai, Y. Liu, X. Z. Shu, G. D. Prestwich, "Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor," *Biomaterials*, **26**, 6054–6067 (2005).
2. D. B. Pike, S. Cai, K. R. Pomraning, M. A. Firpo, R. J. Fisher, X. Z. Shu, G. D. Prestwich, R. A. Peattie, "Heparin-regulated release of growth factors *in vitro* and angiogenic response *in vivo* to implanted hyaluronan hydrogels containing VEGF and bFGF," *Biomaterials*, **27**, 5242–5251 (2006).
3. Unpublished data from G. D. Prestwich, et al, University of Utah, and R. Peattie, et al, University of Oregon.
4. G. D. Prestwich, Y. Liu, M. Serban, B. Yu, X. Z. Shu, and A. Scott, "3-D Culture in Synthetic Extracellular Matrices: New Tissue Models for Drug Toxicology and Cancer Drug Discovery," invited, *Adv. Enz. Res.*, in press (2007).
5. X. Z. Shu, S. Ahmad, Y. Liu, and G. D. Prestwich, "Synthesis and Evaluation of Injectable, In Situ Crosslinkable Synthetic Extracellular Matrices (sECMs) for Tissue Engineering," *J. Biomed Mater. Res. A*, **79A**(4), 901-912 (2006).

Procedure

Rigidity of the hydrogel can be varied either by changing the volume of 1× Extralink 2 Stock Solution used for crosslinking⁴ or by diluting the 1× HyStem-HP and Gelin-S Stock Solutions using PBS or cell culture medium. Diluting these Stock Solutions with PBS or cell culture medium can increase the gelation time.

The following is a procedure to prepare a 2.5 mL batch of hydrogel scaffold.

1. Allow the HyStem-HP, Gelin-S, Extralink 2, and degassed water bottles to come to room temperature.
2. Under aseptic conditions, using a syringe and needle, add 1.0 mL of degassed water (**Cat. No. W3894**) to the HyStem-HP bottle. Repeat for the Gelin-S bottle.
3. Place both bottles horizontally on a rocker or shaker. It will take <30 minutes for the solids to fully dissolve. Warming to 37 °C and/or gently vortexing will speed dissolution. 1× Stock Solutions will be clear and slightly viscous.
4. Under aseptic conditions, using a syringe and needle, add 0.5 mL of degassed water (**Cat. No. W3894**) to the Extralink 2 bottle. Invert several times to dissolve.
5. As soon as possible, but within 2 hours of making the solutions, aseptically mix the HyStem and Gelin-S 1× Stock Solutions together. To mix, pipette back and forth slowly to avoid trapping air bubbles.
6. If adding growth factors/ECM proteins, add sterile growth factors/ECM protein solution to the 1:1 mixture of HyStem and Gelin-S 1× Stock Solutions. Pipette back and forth to mix.
7. If encapsulating cells, resuspend the cell pellet in the 1:1 mixture of HyStem and Gelin-S 1× Stock Solutions. Pipette back and forth to mix.
8. To form the hydrogel, combine the following and mix by pipette: 0.5 mL of 1× Extralink 2 Stock Solution 2.0 mL of HyStem/Gelin-S 1:1 mixture
9. Gelation will occur within ~20 minutes.

HydroMatrix™ Peptide Hydrogel

A synthetic peptide nanofiber scaffold, HydroMatrix offers the precision and control of a synthesized matrix with the natural 3-D architecture of a highly crosslinked peptide hydrogel. The HydroMatrix scaffold self-assembles from fluid precursors into a highly crosslinked peptide 3-D hydrogel, responding to changes in temperature or ionic strength. By adjusting the concentration of the HydroMatrix solution, researchers are able to control the flexibility of the 3-D architecture, and tailor the structure to meet their individual needs. HydroMatrix promotes cell growth and migration, and has been shown to support the proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, and keratinocytes.

- Precision of a highly synthesized matrix allows you to customize and control the scaffold
- Natural 3-D architecture of highly crosslinked peptide hydrogel creates a natural environment for your cells
- Proven proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, and keratinocytes

What makes HydroMatrix superior to other 3-D systems is that it's a fully synthetic material with no possible source of infection or contamination from animal-derived matter, allowing researchers to focus on more important things.

HydroMatrix Protocols and Technical Information

Plating of Cells on HydroMatrix Coated Plates

1. Determine the optimal concentration for the particular application and dilute the stock solution with distilled water. Keep the diluted hydrogel solution on ice until ready to use.
2. Add diluted hydrogel solution to the cell culture well. See **Table 1** for recommended volumes. Induce formation of the gel by adding 1–2 volumes of medium to the side of each well.

Plate Size	Volume of dilute HydroMatrix Solution
96-well plate	7 μ L per well
24-well plate	500 μ L per well
6-well plate	2.4 mL per well

Table 1: Recommended volumes of HydroMatrix per well

3. Incubate the plate at 37 °C for 1 hr to allow the gel to form. Then carefully change the medium twice over 1–2 hrs and allow the gel to incubate at 37 °C during this time. Keep the gelled plate at 37 °C for no more than 8 hrs before use.
4. Add desired number of cells in medium to the top of the hydrogel.

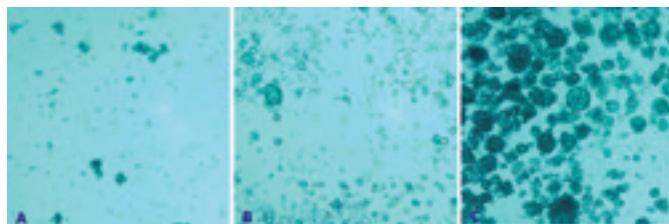


Figure 17. HydroMatrix Peptide Scaffold

Rat neural stem cells (NSC) cultured on three surfaces. NSC grew poorly on tissue culture plastic (A) and slightly better in poly-L-lysine/laminin coated plates (B). NSC demonstrated excellent growth on HydroMatrix peptide hydrogel 0.5% (w/v) (C).

Plating of Cells on HydroMatrix Coated Inserts

1. Determine the optimal concentration for the particular application and dilute the stock solution with distilled water. Keep the diluted hydrogel solution on ice until ready to use.
2. Add enough medium to the lower chamber of each insert so the medium level is just touching the bottom of the insert.
3. Add diluted hydrogel solution to the cell culture insert. See **Table 2** for recommended volumes. Induce formation of the gel by adding 1–2 volumes of medium to the side of each insert.

Insert Size	Volume of dilute HydroMatrix Solution
96-well insert	35 μ L per well
24-well insert	250 μ L per well
6-well insert	1.0 mL per well

Table 2: Recommended volumes of HydroMatrix per insert

4. Incubate the insert at 37 °C for 1 hour to allow the gel to form. Then carefully change the medium 2 times over 1–2 hours and allow the gel to incubate at 37 °C during this time. Keep the gelled insert at 37 °C for no more than 8 hours before use.
5. Add desired number of cells in medium to the top of the hydrogel. Add additional medium below the insert.

Ordering Information		
Cat. No.	Product Description	Size
A6982	HydroMatrix (liquid)	1 mL, 5 mL, 10 mL
H4165	HydroMatrix 96-well plate	1 each
H4040	HydroMatrix 24-well plate	1 each
H3915	HydroMatrix 6-well plate	1 each

MaxGel™ Human ECM

Produced *in vitro*, MaxGel human ECM provides a rich 3-D environment to promote cellular proliferation. MaxGel ECM contains extracellular matrix components including collagens, laminin, fibronectin, tenascin, elastin, and a number of proteoglycans and glycosaminoglycans. The cell-cultured derived ECM effectively reproduces the cooperative interaction of epithelia and mesenchyme during development and in organotypic cell culture of skin. The human MaxGel ECM promotes cell growth and migration and has been shown to support the proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, hepatocytes, and keratinocytes.

MaxGel in Stem Cell Applications

Stem cell research has demonstrated the need for human ECM proteins in cell culture as 3-D support matrices for stem cells. The proteins provide the cues necessary to expand stem cell in culture and to guide them through the process of differentiation into many cell types to treat different diseases, such as Pancreatic islets for diabetes and neuronal cells for repair of neurological disorders.

MaxGel contains reduced amounts of growth factors since it is a derived basement membrane extract cultured *in vitro*, which supports lot-to-lot consistency. Other ECM products are solubilized basement membrane preparations extracted from mouse tumor and include high quantities of endogenous growth factors.

Ordering Information

Cat. No.	Product Description	Size
E0282	MaxGel Human ECM	100 µl, 1 mL
M1073	MaxGel 96-well plate	1 each

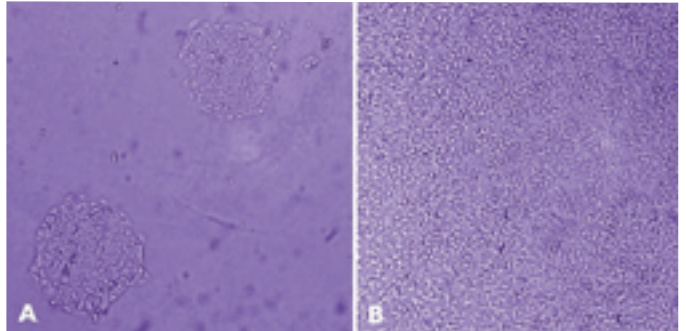


Figure 18. MaxGel enables improved expansion of Adult Keratinocytes

HaCaT cells (derived from human adult skin keratinocytes) were grown for 24 hours after plating on tissue culture plastic (A) and on 1% human ECM (B), which demonstrates that HaCaT cells proliferate better on ECM.

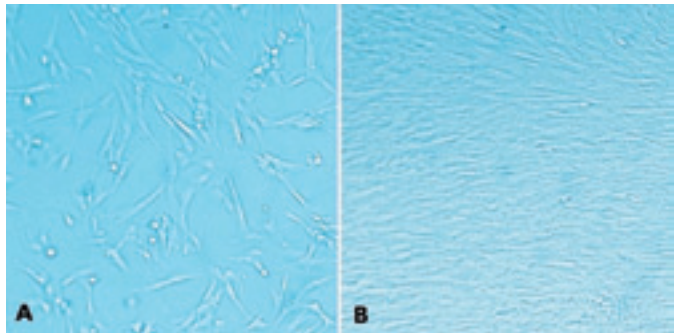


Figure 19. MaxGel enables improved expansion of Fetal Lung Fibroblasts

MRC-5 cells (derived from human fetal lung fibroblasts) were grown for 24 hours after plating on tissue culture plastic (A) and on 1% human ECM (B). As seen with other cells, MRC-5 cells propagate better on ECM.

MaxGel™ Application Data

Human ES Cells Grown on Three Commercial Matrices

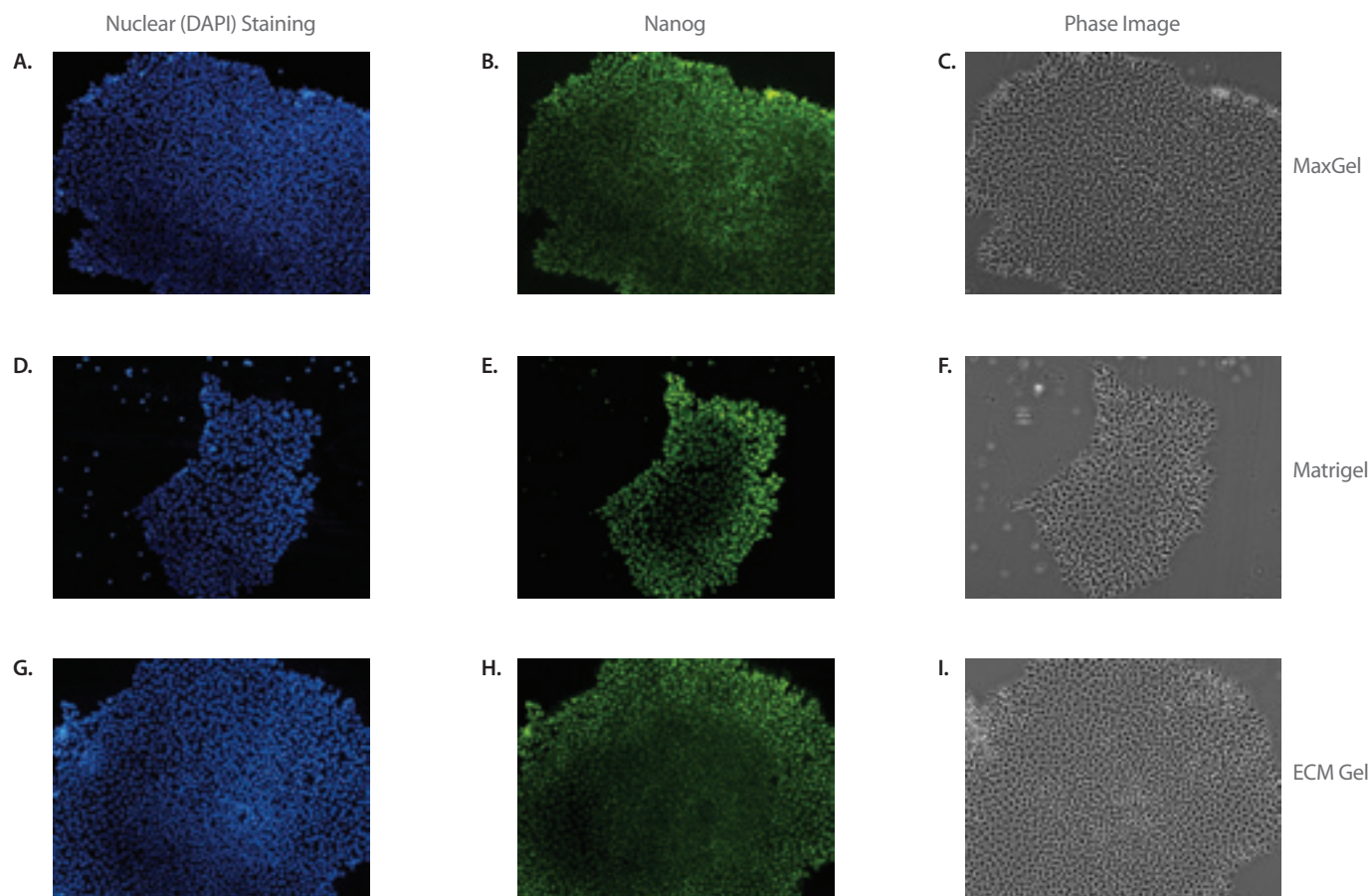


Figure 20. MaxGel human ECM facilitates the attachment and growth of undifferentiated human ES cells. Human Embryonic Stem Cells were cultured on three different extracellular matrices for 11 passages before fixation and immunofluorescent staining of the pluripotency marker, Nanog. Nanog staining was performed with an anti-Nanog monoclonal antibody (Cat. No. N3038) followed by visualization with an anti-mouse FITC conjugated secondary antibody (Cat. No. F2012). Nuclei stained with DAPI (Cat. No. D8417) in blue. Scale bar represents 100 micrometers. All images were taken using a 10x microscope objective. Immunohistochemistry reveals sustained and uniform expression of the pluripotency marker, Nanog, across all three conditions.

- A. Nuclear staining with DAPI.
- B. Anti-Nanog staining with FITC conjugated secondary.
- C. Phase image of a fixed hES colony grown on MaxGel.
- D. Nuclear staining with DAPI.
- E. Anti-Nanog staining with FITC conjugated secondary.
- F. Phase image of a fixed hES colony grown on leading competitor BD's ES qualified Matrigel.
- G. Nuclear staining with DAPI.
- H. Anti-Nanog staining with FITC conjugated secondary.
- I. Phase image of a fixed hES colony grown on ECM gel from Englebreth-Holm-Swarm murine sarcoma (Cat. No. E1270).

MaxGel™ Protocols and Technical Information

Thin Gel Plating Method

A thin gel is used when plating cells on top of a thin layer of gel (requires more basement membrane extract (BME) than the thin coat method). The recommended thin coating volume of BME is 150 $\mu\text{l}/\text{cm}^2$.

1. To prepare a thin gel, dilute the BME solution in cell culture medium. Keep BME on ice until coating.
2. Plate an appropriate volume of diluted BME solution in a tissue culture plate and allow it to incubate for 1–2 hours at 37 °C in a humidified 5% CO₂ incubator.
3. Carefully aspirate the remaining medium.
4. Rinse once with cell culture medium and carefully aspirate the medium again.
5. Air-dry for 30 minutes at room temperature.
6. Plate cells as desired.

Thin Coat Plating Method

Thin coating is used when a slight coating of BME is desired followed by the addition of cells on top of the coated surface. Dilutions for this application can be as high 1:100.

1. To prepare a thin coat, an empirically determined dilution should be performed depending on the particular application. Keep BME on ice until coating.
2. Plate the appropriate volume of diluted BME solution in a tissue culture plate and allow it to incubate for 2–4 hours at 37 °C in a humidified 5% CO₂ incubator.
3. Carefully aspirate the remaining medium.
4. Rinse once with DMEM medium and carefully aspirate the medium again.
5. Air-dry for 30 minutes at room temperature.
6. Plate cells as desired.

Thick Gel Plating Method

The thick gel is used when the cells are to be encapsulated within the gel. The recommended thick coating volume of BME is 300 $\mu\text{l}/\text{cm}^2$. This volume includes the volume that contains the cells.

1. Make a 2× BME solution in the desired cell culture medium, which will be added to a solution containing 2× the desired cell concentration in cell culture medium. Keep BME on ice until coating.
2. Add the 2× BME solution to the 2× cell solution. For example, to make 500 μl of plating solution, add 250 μl of 2× BME and 250 μl of cells at 2× concentration in cell culture medium.
3. Quickly and carefully plate the 1× BME solution containing the cells.

Storage/Stability

Product is stable for >1 year when stored at –20 °C. Storage at –70 °C increases stability. Remove BME from freezer and thaw on ice just prior to use. BME quickly forms a gel when allowed to warm to room temperature, so it is necessary to keep the BME on ice until coating.

Reference

4. Maas-Szabowski, N., et al, Experimental models to analyze differentiation functions of cultured keratinocytes *in vitro* and *in vivo*. *Methods Mol. Biol.*, **2005**; 289: 47–60.

For more information on Sigma 3-D Matrices, visit sigma.com/stemcells3d

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