

Product Information

HyStem™-HP Cell Culture Scaffold Kit for 2.5 ml of hydrogel scaffold

Catalog Number **HYSHP020**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

The HyStem™-HP Cell Culture Scaffold Kit provides an excellent starting point for optimizing the matrix for stem cell culture, where slowly released growth factors are crucial in re-creating a stem cell niche. To affect specific cell performance, growth factors or ECM proteins may be added to the HyStem-HP hydrogel. Unlike animal-derived ECM products, this kit contains three fully chemically defined components, which are nonimmunogenic:

HyStem-HP – a thiol-modified hyaluronan (a major constituent of native ECM), carboxymethyl hyaluronic acid-thiopropionyl hydrazide (CMHA-S, CMHA-DTPH, carboxymethyl hyaluronic acid-DTPH) with heparin-thiopropionyl hydrazide (HP-DTPH). HyStem-HP (CMHA-DTPH with HP-DTPH) contains 99.7 wt% CMHA-DTPH and 0.3 wt% HP-DTPH.

Gelin-S™ – a thiol-modified gelatin (denatured collagen), carboxymethyl gelatin-thiopropionyl hydrazide (GTN-DTPH, carboxymethyl gelatin-DTPH)

Extralink™ – a thiol-reactive crosslinker, polyethylene glycol diacrylate ($M_w = 3,400$ g/mole, PEGDA)

HyStem-HP hydrogels contain thiol-modified heparin, which allows the slow release of growth factors (GF) within an easily customizable environment. Several stem cell types depend on specific ECM components to grow and differentiate. ECM proteins and growth factors are easily incorporated noncovalently into the hydrogel prior to gel formation.

The Gelin-S provides basic cell attachment sites for cell lines and primary cells.¹ Several cell types require specific components of the natural ECM, laminin, collagen, fibronectin, and vitronectin, to grow and differentiate.

The immobilized heparin in the hydrogel mimics the heparan sulfate proteoglycans normally present in the extracellular matrix (ECM). The thiolated heparin ionically binds a wide variety of growth factors and slowly releases them over time. It also helps protect GF from proteolysis.² 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 GF tested (bFGF, VEGF, Ang-1, PDGF, TGF β 1, and KGF) are released at different rates, but over a period of several weeks.^{2,3}

The stem cell culture can be plated on top of the hydrogel for pseudo three dimensional (3D) growth.¹ The hydrogel matrix also provides a basic scaffold for 3D stem cell growth. The stem cells can be encapsulated during crosslinking,⁴ where they attach and grow within the hydrogel. The hydrogel rigidity may be varied to match the stiffness of native tissues.

Components

HyStem-HP	1 × 1 ml
Each bottle contains 10 mg of HyStem-HP and 9.6 mg of phosphate buffered saline (PBS) salts (Catalog Number H2541)	
Gelin-S	1 × 1 ml
Each bottle contains 10 mg of Gelin-S and 9.6 mg of PBS salts (Catalog Number G3673)	
Extralink 2	1 × 0.5 ml
Each bottle contains 10 mg of Extralink and 4.8 mg of PBS salts (Catalog Number E6659)	
Water, degassed	1 × 10 ml
Ready-to-use bottle contains 10 ml of deionized water with 9.6 mg of PBS salts (Catalog Number W3894)	

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Note: Do not uncap the HyStem-HP and Gelin-S bottles since both materials will crosslink in the presence of oxygen. Use a syringe and needle to add degassed water. Prepare 1× Stock Solutions:

HyStem-HP – reconstitute a bottle with 1 ml of degassed water (Catalog Number W3894)

Gelin-S – reconstitute a bottle with 1 ml of degassed water (Catalog Number W3894)

Extralink 2 – reconstitute a bottle with 0.5 ml of degassed water (Catalog Number W3894)

The 1× Stock Solutions will contain 1× phosphate buffered saline (PBS), pH ~7.4.

Storage/Stability

The lyophilized powders are blanketed with argon and under a slight vacuum. They may be stored unopened in the original bottles at –20 °C for up to one year. Do not uncap the HyStem-HP and Gelin-S bottles since both materials will crosslink in the presence of oxygen.

The 1× Extralink 2 Stock Solution may be stored at –20 °C for ~1 month.

Procedure

The 1× Stock Solutions remain liquid at 15–37 °C. The hydrogel is formed when the crosslinking agent, Extralink, is added to a mixture of HyStem-HP and Gelin-S. Gelation occurs in ~20 minutes after all three 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 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 (Catalog Number W3894) to the HyStem-HP bottle. Repeat for the Gelin-S bottle (see Preparation Instructions).
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 (Catalog Number 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.

References

1. Shu, X.Z. et al., 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).
2. Cai, S., et al., Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials*, **26**, 6054-6067 (2005).
3. Pike, D.B. et al., 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).
4. Prestwich, G.D. et al., 3-D Culture in Synthetic Extracellular Matrices: New Tissue Models for Drug Toxicology and Cancer Drug Discovery. *Adv. Enz. Reg.*, **47**, 196-207 (2007).
5. Shu, X.Z. et al., In situ crosslinkable hyaluronan hydrogels for tissue engineering. *Biomaterials*, **25**, 1339-1348 (2004).

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