3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

HyStem®-C Cell Culture Scaffold Kit
for 7.5 ml of hydrogel scaffold

Catalog Number **HYSC020**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The HyStem®-C Cell Culture Scaffold Kit provides an excellent starting point for optimizing the matrix for stem cell culture. It is recommended for cultures, which require a minimal number of cell attachment sites or the addition of other extracellular matrix (ECM) proteins. Unlike animal-derived ECM products, this kit contains three fully chemically defined components, which are nonimmunogenic:

HyStem – a thiol-modified hyaluronan (a major constituent of native ECM), carboxymethyl hyaluronic acid-thiopropanoyl hydrazide (CMHA-S, CMHA-DTPH, carboxymethyl hyaluronic acid-DTPH)

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

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

Hydrogels prepared from these kit components can be customized to fit the growth requirements of the stem cell culture of interest.

The Gelin-S provides basic cell attachment sites for cell lines and primary cells.[1,2] Several cell types require specific components of the natural ECM, laminin, collagen, fibronectin, and vitronectin, to grow and differentiate. Any of these can easily be incorporated noncovalently into the hydrogel prior to gel formation.

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,[3] where they attach and grow within the hydrogel. The hydrogel rigidity may be varied to match the stiffness of native tissues.

Components

HyStem 3 × 1 ml
Each bottle contains 10 mg of HyStem and 9.6 mg of phosphate buffered saline (PBS) salts (Catalog Number H2416)

Gelin-S 3 × 1 ml
Each bottle contains 10 mg of Gelin-S and 9.6 mg of PBS salts (Catalog Number G3673)

Extralink 2 3 × 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 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 – 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 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 (thiol-modified hyaluronan) and Gelin-S (thiol-modified gelatin). 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 crosslinking[4] 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. Sufficient reagents are provided to prepare 3 batches (7.5 ml).

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 (Catalog Number W3894) to the HyStem 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 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. 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. Shu, X.Z. et al., Disulfide-crosslinked Hyaluronan-Gelatin Hydrogel Films: A Covalent Mimic of the Extracellular Matrix for *In Vitro* Cell Growth. Biomaterials, **24**, 3825-3834 (2003).
3. 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).
4. Vanderhooft, J. et al., Rheological Properties of CrossLinked Hyaluronan-Gelatin Hydrogels for Tissue Engineering. Macromol. Biosci., **9**, 20-28 (2009).
5. Shu, X.Z. et al., *In Situ* Crosslinkable Hyaluronan Hydrogels for Tissue Engineering. Biomaterials, **25**, 1339-1348 (2004).

HyStem, Gelin-S, and Extralink are registered trademarks of Glycosan BioSystems, Inc.

JW,MAM 11/11-1