Product Information Sheet

HyStem®-C Cell Culture Scaffold Kit

Trial Kit, For 2.5 mL of hydrogel scaffold solution

HYSC010

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®2 – a thiol-reactive crosslinker, polyethylene glycol diacrylate (MW = 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® - 1 x 1 mL
Each bottle contains 10 mg of HyStem® and 9.6 mg of phosphate buffered saline (PBS) salts
(Cat. No. H2416)

Gelin-S® - 1 x 1 mL
Each bottle contains 10 mg of Gelin-S® and 9.6 mg of PBS salts (Cat. No. G3673)

Extralink® 2 - 1 x 0.5 mL
Each bottle contains 10 mg of Extralink® and 4.8 mg of PBS salts (Cat. No. E6659)

Water degassed - 1 x 10 mL
Ready-to-use bottle contains 10 mL of deionized water with 9.6 mg of PBS salts (Cat. No. W3894)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

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 1x Stock Solutions:

- HyStem® – reconstitute a bottle with 1 mL of degassed water (Cat. No. W3894)
- Gelin-S® – reconstitute a bottle with 1 mL of degassed water (Cat. No. W3894)
- Extralink® 2 – reconstitute a bottle with 0.5 mL of degassed water (Cat. No. W3894)
- The 1x Stock Solutions will contain 1x phosphate buffered saline (PBS), pH ~7.4.

Storage/Stability

Storage Temperature –20 °C

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

Procedure

The 1x 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 1x Extralink® 2 Stock Solution used for crosslinking[4] or by diluting the 1x 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 (see Preparation Instructions).
3. Place both bottles horizontally on a rocker or shaker. It may take ~ 60 minutes for some components to fully dissolve. Warming to ≤ 37 °C and/or gently vortexing will speed dissolution. 1x 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® 1x 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® 1x 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® 1x Stock Solutions. Pipette back and forth to mix.
8. To form the hydrogel, combine the following and mix by pipette:
    - 0.5 mL of 1x Extralink® 2 Stock Solution
    - 2.0 mL of HyStem®/Gelin-S® 1:1 mixture
9. Gelation will begin within ~ 15 minutes and full gelation will occur by ~ 90 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).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.