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# Product Information

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

Catalog Number **HYS020**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

The HyStem® Cell Culture Scaffold Kit is optimal for culturing stem cells whose natural environment is rich in hyaluronic acid (HA) and applications requiring attachment factor optimization. It can be customized by adding extracellular matrix (ECM) proteins[1] to provide attachment site and/or differentiation signals. Unlike animal-derived ECM products, this kit contains two fully chemically defined, animal-free 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)

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

Since HyStem hydrogels are composed of only HyStem and Extralink, they do not support cell attachment.[2] If plating cells on the surface of the hydrogels, then ECM proteins can be non-covalently incorporated into the hydrogel to promote cell attachment. Because the ECM proteins can also provide differentiation signals as well as attachment signals, the appropriate type to use must be determined.

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.[4]

### 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.

### Components

| | |
|---|---|
| HyStem<br>Each bottle contains 10 mg of HyStem and 9.6 mg of phosphate buffered saline (PBS) salts (Catalog Number H2416) | 6 × 1 ml |
| Extralink 1<br>Each bottle contains 5 mg of Extralink and 4.8 mg of PBS salts (Catalog Number E6534) | 3 × 0.5 ml |
| Water, degassed<br>Ready-to-use bottle contains 10 ml of deionized water with 9.6 mg of PBS salts (Catalog Number W3894) | 1 × 10 ml |

### Preparation Instructions

Note: Do not uncap the HyStem S bottles since the material 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)

Extralink 1 – 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 bottles since the material will crosslink in the presence of oxygen.

The 1× Extralink 1 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 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[5] 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 (Catalog Number W3894) to each HyStem bottle (see Preparation Instructions).
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 (Catalog Number 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 1× HyStem Stock Solution
9. Gelation will occur within <20 minutes.

**References**


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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. Ghosh, K. et al., Cell Adaptation to a Physiologically Relevant ECM Mimic with Different Viscoelastic Properties. Biomaterials, **28**, 671-679 (2007).
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