

## HyStem<sup>®</sup> 12.5 mL Kit

### THIOL-MODIFIED HYALURONAN HYDROGEL KIT

Catalog Number #GS1004

#### OVERVIEW

The HyStem Hydrogel Kit is composed of Glycosil<sup>®</sup> (thiol-mod-ified hyaluronic acid), Extralink<sup>®</sup>-Lite (PEGDA, polyethylene glycol diacrylate), and degassed, deionized water (DG Water). A solution of Glycosil forms a transparent hydrogel when mixed with Extralink-Lite. Glycosil and Extralink-Lite are packaged as lyophilized solids that are blanketed by nitrogen and under a slight vacuum.

#### CELL ATTACHMENT

The HyStem hydrogel system provides a viscoelastic matrix of variable rigidity that supports the expansion of stem cells (human embryonic, CD34+, and hepatic progenitors have been tested to date). HyStem hydrogels do not support surface cell attachment. Cells must be either encapsulated within the hydro-gel, or extracellular matrix (ECM) proteins or peptides may be mixed with the Glycosil prior to crosslinking to provide attach-ment signals and allow for cells to be plated on the hydrogel surface. However, the type of ECM protein added depends upon the cell type and the desired outcome (expansion without differentiation or with differentiation).

#### STORAGE

**Glycosil:** Store Glycosil in original vials at -20°C for up to one year. Do not uncap the Glycosil vials since material will crosslink in the presence of oxygen. Use a syringe to add DG Water and remove product from the vials.

**Extralink-Lite:** Store Extralink-Lite in the original vial at -20°C for up to one year. Reconstituted solutions can be stored at -20°C for ~ one month.

Note: It is recommended to reconstitute each vial in its entirety.

#### INSTRUCTIONS FOR USE

Glycosil and Extralink-Lite solutions are prepared by dissolving the lyophilized solids in the DG Water. When reconstituted, the materials will be in 1x phosphate buffered saline (PBS), pH ~7.4. Glycosil vials contain 50 mg of material and when reconstituted according to instruction will produce a 1% (w/v) solution. Extra-link-Lite vials contain 12.5 mg of diacrylated PEG and when reconstituted according to instructions will produce a ~0.5% (w/v) solution. HyStem hydrogels (6 x 1.25 mL = 7.5 mL) should be prepared in the following manner:

1. Allow the Glycosil, Extralink-Lite, and DG Water to come to room temperature.
2. Under aseptic conditions using a syringe and needle add 5.0 mL of DG Water to the Glycosil vial.
3. Place the vial horizontally on a rocker or shaker. It will take ~40 minutes for the solids to fully dissolve. Warming to not more than 37 °C and/or gently vortexing will speed dissolution. The solution will be clear and slightly viscous.
4. Under aseptic conditions using a syringe and needle add 2.5 mL of DG Water to the Extralink-Lite vial. Invert several times to dissolve.
5. If encapsulating cells, resuspend cell pellet in 10 mL of Glycosil. Pipette back and forth to mix.
6. To form the hydrogel, add Extralink-Lite to the Glycosil mix in a 1:4 volume ratio (2.5 mL Extralink-Lite to 10 mL Glycosil) mix by pipette.
7. If encapsulating cells, allow solution to react for 10 minutes then mix again by pipette to ensure even distribution of cells.
8. Gelation will occur within ~30 minutes.
9. Freeze unused Extralink-Lite at -20°C for use with another Glycosil vial