HyStem® Hydrogel Kit with PEGSSDA, 7.5 mL

Catalog Number: GS311P

OVERVIEW

The HyStem® Hydrogel Kit with PEGSSDA is composed of Glycosil® (thiol-modified hyaluronic acid), PEGSSDA™ (disulfide-containing polyethylene glycol diacrylate), and degassed, deionized water (DG Water). A solution of Glycosil forms a transparent hydrogel when mixed with PEGSSDA. Glycosil and PEGSSDA 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 hydrogel, or extracellular matrix (ECM) proteins or peptides may be mixed with the Glycosil prior to crosslinking to provide attachment 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 both materials will crosslink in the presence of oxygen. Use a syringe to add DG Water and remove product from the vials.

PEGSSDA: Store PEGSSDA in the original vial at -20°C for up to one year.

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

INSTRUCTIONS FOR USE

Glycosil and PEGSSDA solutions are prepared by dissolving the lyophilized solids in the DG Water. When reconstituted, they will be in 1X phosphate buffered saline (PBS), pH ~7.4. Glycosil vials contain 10 mg of material and when reconstituted according to instruction will produce a 1% (w/v) solution. PEGSSDA vials contain 10 mg of material and when reconstituted according to instructions will produce a 2% (w/v) solution.

HyStem hydrogels (3 X 2.5 mL = 7.5 mL) should be prepared in the following manner:

1. Allow the Glycosil, PEGSSDA, and DG Water vials to come to room temperature.
2. Under aseptic conditions and using a syringe add 1.0 mL of DG Water to the Glycosil vial.
3. Place vials 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 gentle vortexing will speed dissolution. Solutions will be clear and slightly viscous.
4. Under aseptic conditions and using a syringe add 0.5 mL of DG Water to the PEGSSDA vial. Invert several times to dissolve.
5. As soon as possible, but within 2 hours of making the solutions, mix two vials of Glycosil. To mix, pipette back and forth to mix.
6. If encapsulating cells, resuspend cell pellet in 2.0 mL of Glycosil. Pipette back and forth to mix.
7. To form the hydrogel, add PEGSSDA to the Glycosil mix in a 1:4 volume ratio (0.5 mL PEGSSDA to 2.0 mL Glycosil) mix by pipette.
8. If encapsulating cells, allow solution to react for 10 minutes then mix again by pipette to ensure even distribution of cells.
9. Gelation will occur within ~30 minutes.

DISSOLUTION

Dissolution of gels with cells on top and encapsulated (gel volume of 0.6 mL) in a 24 well plate. The following procedure was optimized particularly for the aforementioned gel geometry. Dissolution of gels with alternate geometry and/or volumes may require adjustments to the protocol. In general, for dissolution add at least twice the gel volume of N-Acetyl-L-Cysteine.

1. Make up the appropriate amount of 40mM N-Acetyl-L-Cysteine in 1X PBS or media and pH to 7.4.

2. Add 2 mL of 40mM N-Acetyl-L-Cysteine to the top of each gel and let sit at 37°C for 1 hour. Agitation by orbital shaking will help decrease dissolution time.

3. Confirm dissolution by pipetting solution in well up and down, observing any remaining gel. If needed, allow another 30 minutes for complete gel dissolution.
4. Remove liquid from well and place in conical centrifuge tube. If necessary, add PBS to a total of 5mL of liquid.
5. Centrifuge at 1000 RPM for 5 minutes.
6. Aspirate off liquid and process cells as desired.

Note: Each kit component has been manufactured under aseptic conditions and tested for bacteria and fungus.

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