ADVANCED BIOMATRIX
A BICO COMPANY

HyStem® Kits

THIOL-MODIFIED HYALURONAN HYDROGEL KIT

Catalog Number: **#GS310F (2.5 mL)** **#GS311F (7.5 mL)** **#GS1004F (12.5 mL)**

OVERVIEW

HyStem® kits are based on cross-linking thiol-modified hyaluronan technology. Hyaluronic acid is a naturally occurring component of the extracellular matrix found in connective, epithelial, and neural tissues. With HyStem®, researchers can create customizable 3D hydrogels for culturing cells whose natural environment is rich in hyaluronic acid. The HyStem® Hydrogel Kit includes:

- Glycosil® (thiol-modified hyaluronic acid)
- Extralink®-Lite (PEGDA, polyethylene glycol diacrylate)
- Buffer A (Neutralization Solution)
- Buffer B (1X PBS pH 7.4)

Hystem® Kit components form a transparent hydrogel when mixed. Components, except for Buffer A and Buffer B, are packaged as lyophilized solids that are blanketed by nitrogen and under a slight vacuum for long term storage.

#GS310F (2.5 mL)	# of Units	Material Amount Per Vial	Reconstitution Volume Per Vial
Glycosil – GS222F	2	10 mg	1.0 mL
Extralink-Lite – GS3009F	1	3.75 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

#GS311F (7.5 mL)	# of Units	Material Amount Per Vial	Reconstitution Volume Per Vial
Glycosil – GS222F	6	10 mg	1.0 mL
Extralink-Lite – GS3009F	3	3.75 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

#GS1004F (12.5 mL)	# of Units	Material Amount Per Vial	Reconstitution Volume Per Vial
Glycosil – GS220F	2	50 mg	5.0 mL
Extralink-Lite – GS3008F	1	18.75 mg	2.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

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 encapsulated within the hydrogel. 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). Hystem®-C and Hystem®-HP kits support cell attachment and can be found at AdvancedBioMatrix.com

STORAGE

Glycosil: Store at -20 or 4°C for up to one year. Reconstituted solutions must be used same day and cannot be refrozen.

Extralink-Lite: Store at -20 or 4°C for up to one year. Reconstituted solutions can be stored at -20°C for one month.

Buffer A: Store at 4°C or RT for up to one year.

Buffer B: Store at 4°C or RT for up to one year.

INSTRUCTIONS FOR USE

Glycosil and Extralink-Lite solutions are prepared by dissolving the lyophilized solids with reconstitution buffers. When reconstituted, Glycosil and Extralink-Lite will be in 1X phosphate buffered saline (PBS) at a pH of ~7.4. When reconstituted according to instructions, the kits will be able to produce 2.5, 7.5 or 12.5 mL of material to form 3D hydrogels.

1) Allow kit components to come to room temperature.

2) Under aseptic conditions, using a syringe and needle, add the following buffers to each Hystem component. Follow reconstitution chart below. If vial stopper is removed during reconstitution, minimize exposure to oxygen to avoid potential auto-crosslinking. **DO NOT WEIGH OUT COMPONENTS OR USE ANOTHER BUFFER DURING RECONSITUTION.**

Kit Components	Buffer to add per vial
Glycosil – GS222F	1.0 mL of Buffer A
Glycosil – GS220F	5.0 mL of Buffer A
Extralink-Lite – GS3009F	0.5 mL of Buffer B
Extralink-Lite – GS3008F	2.5 mL of Buffer B

3) Immediately vortex each vial for a few seconds after the addition of Buffer A or Buffer B. Place vials horizontally on a rocker or shaker. Quickly vortex samples every 15 minutes. It may take 1 hour for some components to fully dissolve. Warming to 37 °C and gently vortexing will speed dissolution. Components will be clear and slightly viscous.

4) A 3D hydrogel is formed when Extralink-Lite is added to Glycosil in a 1:4 volume ratio. E.g., 0.25 mL of Extralink-Lite to 1.0 mL of Glycosil.

5) Mix components together by pipette.

6) If encapsulating cells, resuspend cell pellet in Glycosil solution *prior* to the addition of Extralink-Lite. Pipette back and forth to mix.

7) After mixing all components together, wait for 5 minutes, then mix again by pipette to ensure even distribution of cells.

8) Dispense HyStem into desired well-plate. Gelation will begin within ~10 minutes and full gelation will occur by ~90 min.

Additional Hystem® information, white papers, applications, references, and certificates, can be found by visiting at www.AdvancedBioMatrix.com