

HyStem[®]- C 2.5 mL, 7.5 mL and 12.5 mL Kits
 THIOL-MODIFIED HYALURONAN AND GELATIN HYDROGEL KIT
 Catalog Number: **#GS312F (2.5 mL) #GS313F (7.5 mL) #GS1005F (12.5 mL)**

OVERVIEW

HyStem-C[®] 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-C[®], researchers can create customizable 3D hydrogels for culturing cells whose natural environment is rich in hyaluronic acid. The HyStem[®]-C Hydrogel Kit includes:

- Glycosil[®] (thiol-modified hyaluronic acid)
- Gelin-S[®] (thiol-modified gelatin)
- Extralink[®] (PEGDA, polyethylene glycol diacrylate)
- Reconstitution Buffer (PBS)

HyStem[®]-C Kit components form a transparent hydrogel when mixed. Components, except for the reconstitution buffer, are packaged as lyophilized solids that are blanketed by nitrogen and under a slight vacuum for long term storage.

Kit Components #GS312F (2.5 mL)	Units Per Kit	Material Amount Per Vial	Reconstitution Volume Per Vial
Glycosil – GS222F	1	10 mg	1.0 mL
Gelin-S – GS231F	1	10 mg	1.0 mL
Extralink – GS3007F	1	5 mg	0.5 mL
Reconstitution Buffer GS250F	1	10 mL	-

Kit Components #GS313F (7.5 mL)	Units Per Kit	Material Amount Per Vial	Reconstitution Volume Per Vial
Glycosil – GS222F	3	10 mg	1.0 mL
Gelin-S – GS231F	3	10 mg	1.0 mL
Extralink – GS3007F	3	5 mg	0.5 mL
Reconstitution Buffer GS250F	1	10 mL	-

Kit Components #GS1005F (12.5 mL)	Units Per Kit	Material Amount Per Vial	Reconstitution Volume Per Vial
Glycosil – GS220F	1	50 mg	5.0 mL
Gelin-S – GS230F	1	50 mg	5.0 mL
Extralink – GS3006F	1	25 mg	2.5 mL
Reconstitution Buffer GS251F	1	20 mL	-

CELL ATTACHMENT

The HyStem[®]-C 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[®]-C hydrogels support surface cell attachment through a thiol-modified ECM component called Gelin-S. HyStem[®]-C allows cells to be either encapsulated within the hydrogel or attached on the surface.

STORAGE

Glycosil / Gelin-S: Store at -20°C for up to one year.

Reconstituted solutions must be used same day and cannot be refrozen.

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

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

INSTRUCTIONS FOR USE

Glycosil, Gelin-S, and Extralink solutions are prepared by dissolving the lyophilized solids with the reconstitution buffer. When reconstituted, Glycosil, Gelin-S and Extralink will be in 1X phosphate buffered saline (PBS) at a pH of ~7.4. When reconstituted according to instructions, this kit 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 for 1 hour.
- 2) Reconstitute kit components using reconstitution buffer with a syringe and needle. If vial stopper is removed during reconstitution, minimize exposure to oxygen to avoid potential autocrosslinking. Follow reconstitution chart below.

Kit Components	Reconstitution Buffer to Add Per Vial
Glycosil – GS222F	1.0 mL
Glycosil – GS220F	5.0 mL
Gelin-S – GS231F	1.0 mL
Gelin-S – GS230F	5.0 mL
Extralink – GS3007F	0.5 mL
Extralink – GS3006F	2.5 mL

- 3) Immediately vortex each vial for a few seconds after the addition of reconstitution water. Place vials horizontally on a rocker or shaker. Quickly vortex samples every 15 minutes. It may take > 2 hours 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 is added to Glycosil and Gelin-S in a 1:2:2 volume ratio.
 - a) For 2.5 mL and 7.5 mL kits: 0.25 mL Extralink to 0.5 mL Glycosil to 0.5 mL Gelin-S. Mix Glycosil and Gelin-S together prior to the addition of Extralink.

For 12.5 mL kits: 2.5 mL Extralink to 5.0 mL Glycosil to 5.0 mL Gelin-S. Mix Glycosil and Gelin-S together prior to the addition of Extralink.

 - b) Mix by pipette.

 - c) If encapsulating cells, resuspend cell pellet in Glycosil + Gelin-S mixture *prior* to the addition of Extralink. Pipette back and forth to mix.

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

- 5) Dispense into desired well-plate. Gelation will begin within ~15 minutes and full gelation will occur by ~90 minutes.

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