

**HyStem®- HP 2.5 mL, 7.5 mL and 12.5 mL Kits**  
THIOL-MODIFIED HYALURONAN, GELATIN AND HEPARIN HYDROGEL KIT  
Catalog Number: **#GS314F (2.5 mL) #GS315F (7.5 mL) #GS1006F (12.5 mL)**

**OVERVIEW**

HyStem®-HP 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®-HP, researchers can create customizable 3D hydrogels for culturing cells whose natural environment is rich in hyaluronic acid. The immobilized heparin in the HyStem®-HP hydrogel mimics the heparin sulfate proteoglycans normally present in the extracellular matrix. The Heparin is utilized for controlled growth factor release. The HyStem®-HP Hydrogel Kit includes:

- Heprasil® (thiol-modified hyaluronic acid and heparin)
- Gelin-S® (thiol-modified gelatin)
- Extralink® (PEGDA, polyethylene glycol diacrylate)
- Reconstitution Buffer (PBS)

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

**CELL ATTACHMENT**

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

**STORAGE**

**Heprasil / 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.

**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**

Heprasil, Gelin-S, and Extralink solutions are prepared by dissolving the lyophilized solids with the reconstitution buffer. When reconstituted, Heprasil, 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 the reconstitution buffer with a syringe and needle. If vial stopper is removed during reconstitution, minimize exposure to oxygen to avoid potential autocrosslinking. **DO NOT WEIGH OUT COMPONENTS OR USE ANOTHER BUFFER DURING RECONSTITUTION.**

Kit Components #GS314F (2.5 mL)	Units Per Kit	Material Amount Per Vial	Reconstitution Volume Per Vial
Heprasil – GS217F	1	10 mg	1.0 mL
Gelin-S – GS231F	1	10 mg	1.0 mL
Extralink – GS3007F	1	5 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

Kit Components #GS315F (7.5 mL)	Units Per Kit	Material Amount Per Vial	Reconstitution Volume Per Vial
Heprasil – GS217F	3	10 mg	1.0 mL
Gelin-S – GS231F	3	10 mg	1.0 mL
Extralink – GS3007F	3	5 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

Kit Components #GS1006F (12.5 mL)	Units Per Kit	Material Amount Per Vial	Reconstitution Volume Per Vial
Heprasil – GS215F	1	50 mg	5.0 mL
Gelin-S – GS230F	1	50 mg	5.0 mL
Extralink – GS3006F	1	25 mg	2.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

Kit Components	Buffer to Add Per Vial
Heprasil – GS217F	1.0 mL
Heprasil – GS215F	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 the reconstitution buffer. 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 Heprasil and Gelin-S in a 1:2:2 volume ratio.
- 5) Mix by pipette.
- 6) If encapsulating cells, resuspend cell pellet in Heprasil + Gelin-S mixture *prior* to the addition of Extralink. 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 into desired well-plate. Gelation will begin within ~10 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](http://www.AdvancedBioMatrix.com)