

HyStem®- HP 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 (ECM) 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 ECM. 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)
- Buffer A (Neutralization Solution)
- Buffer B (1X PBS pH 7.4)

HyStem®-HP 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.

#GS314F (2.5 mL)	# of Units	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	7.5 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

#GS315F (7.5 mL)	# of Units	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	7.5 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

#GS1006F (12.5 mL)	# of Units	Material Amount Per Vial	Reconstitution Volume Per Vial
Heprasil – GS215F	1	50 mg	1.0 mL
Gelin-S – GS230F	1	50 mg	1.0 mL
Extralink – GS3006F	1	37.5 mg	0.5 mL
Buffer A – GS260F	1	10 mL	-
Buffer B – GS250F	1	10 mL	-

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 or 4°C for up to one year. Reconstituted solutions must be used same day and cannot be refrozen.

Extralink: 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

Heprasil, Gelin-S, and Extralink solutions are prepared by dissolving the lyophilized solids with the reconstitution buffers. 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.
- 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 RECONSTITUTION.**

Kit Components	Buffer to add per vial
Heprasil – GS217F	1.0 mL of Buffer A
Heprasil – GS215F	5.0 mL of Buffer A
Gelin-S – GS231F	1.0 mL of Buffer B
Gelin-S – GS230F	5.0 mL of Buffer B
Extralink – GS3007F	0.5 mL of Buffer B
Extralink – GS3006F	2.5 mL of Buffer B

- 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 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 is added to Heprasil and Gelin-S in a 1:2:2 volume ratio. E.g., 0.25 mL Extralink, 0.5 mL Heprasil, and 0.5 mL Gelin-S
- 5) Mix components together by pipette. It is recommended to mix Heprasil and Gelin-S together prior to the addition of Extralink.
- 6) If encapsulating cells, resuspend cell pellet in Heprasil + Gelin-S solution *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 HyStem-HP 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 our visiting at www.AdvancedBioMatrix.com