

HyStem® Hydrogel Stiffness Variation (12.5 mL Kit)

This protocol describes how to vary the stiffness of hydrogels prepared with a 12.5 mL HyStem® Hydrogel Kit, which contains:

- Glycosil® (thiol-modified hyaluronic acid)
- Gelin-S® (thiol-modified gelatin)
- Extralink® (PEGDA, polyethylene glycol diacrylate)
- Buffer A and Buffer B reconstitution buffers

If desired, each component can also be ordered in bulk.

Hydrogel stiffness is primarily controlled by Extralink concentration and Gelin-S concentration. These adjustments also influence gel properties such as:

- Number of attachment sites
- Overall density
- Gelation time (stiffer gels generally solidify more quickly than softer ones)

Required Materials

- HyStem®, HyStem-C®, or HyStem-HP® Hydrogel Kit

Notes

- Extralink concentration is varied by adjusting the reconstitution volume with Buffer B.
- Gelin-S concentration is similarly varied by changing the reconstitution volume.
- Both parameters can be tuned independently or together to achieve the desired stiffness.

Procedure

1. Reconstitute Glycosil and Gelin-S vials according to kit instructions.
2. Adjust Extralink concentration by reconstituting with different amounts of Buffer B (see Table 1).

Table 1. Adjusting Extralink Concentration

<u>Condition</u>	<u>Buffer B (mL)</u>	<u>Extralink Concentration</u>	<u>Approx. Gelation Time (min)</u>
A (Stiffer)	0.625	6.0%	1–3
B	1.25	3.0%	4–9
C	2.50	1.5%	20
D	5.00	0.75%	30–40
E (Softer)	10.00	0.375%	50–80

3. Plating cells on top of hydrogel
 - Mix equal parts Glycosil and Gelin-S.
 - Add Extralink at a 1:4 ratio (Extralink : Glycosil + Gelin-S).
 - Example: 0.5 mL Glycosil + 0.5 mL Gelin-S, then add 0.25 mL Extralink.
 - Pipette immediately into wells of a prepared tissue culture plate.
4. Encapsulating cells within hydrogel
 - Resuspend the cell pellet in equal parts Glycosil and Gelin-S.
 - Add Extralink at a 1:4 ratio (Extralink : Glycosil + Gelin-S).
 - Example: 0.5 mL Glycosil + 0.5 mL Gelin-S + cells, then add 0.25 mL Extralink.

- *Note: Adding extra media will reduce stiffness.*
 - Pipette immediately into cell culture inserts.
5. Adjust Gelin-S concentration by varying reconstitution volume with Buffer B (see Table 2).

Table 2. Adjusting Gelin-S Concentration

<u>Condition</u>	<u>Buffer B (mL)</u>	<u>Gelin-S Concentration</u>
A (Stiffer)	2.5	2%
B (Standard)	5.0	1%
C (Softer)	10.0	0.5%

Variations

- Extralink and Gelin-S concentrations can be adjusted simultaneously to fine-tune stiffness.
- HyStem® or HyStem-HP® kits may be used in place of HyStem-C.