



ECM Protein Incorporation

This protocol is outlined for incorporating laminin into a 12.5 mL HyStem[®]-C kit. However, it can be easily adapted to HyStem[®] and HyStem[®]-HP kits of any size, or for other ECM proteins. HyStem-C includes cellular attachment sites in the Gelin-S but additional ECM proteins are needed for some cell types. HyStem hydrogel kits not containing Gelin-S will not support any attachment without additional factors.

Glycosil[®], Gelin-S[®], and Extralink[®] solutions are prepared by dissolving the lyophilized solids in DG Water. When reconstituted, they will be in phosphate-buffered saline (PBS) pH ~7.6. HyStem[®]-C hydrogels (12.5 mL) with laminin can be prepared in the following manner:

Required Materials:

One HyStem, HyStem-C, or HyStem-HP hydrogel kit
ECM Protein

Steps

1. Remove Glycosil, Gelin-S, and Extralink vials from the -20° C freezer and heat them to 37 °C (~30 minutes).
2. Under aseptic conditions and using a syringe with the exact amount of liquid, add 5.0 mL of DG Water to the Glycosil vial. Repeat for the Gelin-S vial.
3. Place both vials horizontally on a rocker at 37 °C (for maximum mixing).
It will take < 30 minutes for the solids to fully dissolve. Solutions will be clear and slightly viscous.

Note: Vigorous shaking will speed up dissolving time.

4. Under aseptic conditions and using a syringe with the exact amount of liquid, add 2.5 mL of DG Water to the Extralink vial. Invert several times to dissolve.
5. As soon as possible, but within four hours of making the solutions, mix equal volumes of Glycosil and Gelin-S. Pipette or invert to mix.
6. Add 1.1 mL of pH neutral ECM protein to 10 mL of Glycosil + Gelin-S. Mix thoroughly.
7. If encapsulating cells, add 1.0 mL of cells to 11.1 mL of Glycosil + Gelin-S + ECM. Mix thoroughly.
8. To form the hydrogel, add Extralink to the Glycosil + Gelin-S + ECM mix in a 1:4 volume ratio (2.5 mL Extralink to 10.0 mL Glycosil + Gelin-S).

Note: Gelation will occur in ~20 minutes.



Protocol Variations

The amount of ECM can be increased or decreased.

The ECM source can be varied – collagen, fibronectin, laminin, vitronectin, etc...

The amount of cells encapsulated can be increased or decreased.

Cells can be plated on top of the hydrogels instead of being encapsulated.

Gelin-S can be left out entirely and hydrogels can be made with Glycosil, Extralink, and the desired ECM. Note that cells do not attach to Glycosil alone, so if no Gelin-S is used, then an ECM must be added in order for cells to attach.

Other kits can be used instead of the HyStem-C Hydrogel Kit.