

ECM Protein Incorporation into HyStem® Hydrogels

This protocol outlines incorporation of laminin into a 12.5 mL HyStem®-C kit, but it can be adapted for HyStem® or HyStem®-HP kits of any size, as well as other ECM proteins.

HyStem-C contains inherent cell-attachment sites via Gelin-S®. For certain cell types, additional ECM proteins are required. HyStem kits that do not include Gelin-S will not support cell attachment unless an ECM protein is added.

Required Materials

- HyStem®, HyStem-C®, or HyStem-HP® hydrogel kit
- ECM protein of choice (e.g., laminin, collagen, fibronectin, vitronectin, etc.)

Notes

- Glycosil® is reconstituted with Buffer A.
- Gelin-S® and Extralink® are reconstituted with Buffer B.
- After reconstitution, solutions are in PBS, pH ~7.4.
- Gelation begins within 10–15 minutes after adding Extralink.
- Vertexing or vigorous shaking accelerates dissolution, but gentle mixing is preferred for consistency.

Procedure

1. Remove HyStem kit components from the refrigerator and warm to 37 °C (~30 minutes).
2. Under aseptic conditions:
 - Add 5.0 mL Buffer A to the Glycosil vial.
 - Add 5.0 mL Buffer B to the Gelin-S vial.
3. Place both vials horizontally on a rocker at 37 °C until dissolved (<60 minutes). Solutions should be clear and slightly viscous.
4. Add 2.5 mL Buffer B to the Extralink vial. Invert several times to dissolve.
5. Within 4 hours of reconstitution, mix equal volumes of Glycosil and Gelin-S. Pipette or gently invert to mix.
6. Add 1.1 mL of pH-neutral ECM protein to 10 mL of Glycosil + Gelin-S. Mix thoroughly.
7. *(Optional, for encapsulation)* Add 1.0 mL of cell suspension to 11.1 mL of Glycosil + Gelin-S + ECM. Mix thoroughly.
8. To initiate gelation, add Extralink to the Glycosil + Gelin-S + ECM mixture at a 1:4 volume ratio (e.g., 2.5 mL Extralink to 10.0 mL Glycosil + Gelin-S).

Protocol Variations

- Adjust the amount of ECM protein as needed.
- Use alternative ECM proteins: collagen, fibronectin, laminin, vitronectin, etc.
- Cell density can be increased or decreased when encapsulating.
- Cells can be plated on top of the hydrogels instead of being encapsulated.
- Prepare hydrogels with Glycosil + Extralink + ECM only (omit Gelin-S).
 - *Note: Cells will not attach to Glycosil alone. An ECM protein is required for attachment if Gelin-S is excluded.*
- Compatible with all HyStem®, HyStem-C®, and HyStem-HP kits.