

## Fluorescent Labeling of HyStem®-C Hydrogels

This protocol describes how to label HyStem®-C hydrogels with fluorescent dyes such as Alexa Fluor® 350 C5-maleimide (blue) or Alexa Fluor® 488 C5-maleimide (green). Any thiol-reactive C5-maleimide fluorophore may be used. The procedure below yields 1.25 mL of labeled HyStem-C hydrogel.

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### Required Materials

- HyStem®-C Hydrogel Kit
- Alexa Fluor® 350 C5-maleimide (blue)
- Alexa Fluor® 488 C5-maleimide (green)

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### Notes

- Alexa Fluor® 350 C5-maleimide: intense blue fluorescence; excitation ~346 nm; emission ~442 nm (slightly shorter than AMCA/AMCA-X at ~448 nm).
- Alexa Fluor® 488 C5-maleimide: bright green fluorescence; spectrally similar to fluorescein; pH-independent between pH 4–10.
- Maleimides optimally react with thiols at pH ~7.0.
- Fluorophores are light-sensitive and unstable in aqueous solution. Prepare only as much stock as needed for same-day use.

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### Procedure

1. Remove HyStem-C kit components from the refrigerator and warm to 37 °C (~30 min).
2. Prepare a stock solution of Alexa Fluor® dye:
  - Dissolve 0.025 mg Alexa Fluor® 350 C5-maleimide in 10 µL water.
  - If using another fluor, confirm water solubility and check manufacturer guidelines for proper preparation.
3. Under aseptic conditions:
  - Add 1.0 mL Buffer A to the Glycosil vial.
  - Add 1.0 mL Buffer B to the Gelin-S vial.
4. Place both vials horizontally on a rocker at 37 °C until dissolved (<60 min). Solutions should be clear and slightly viscous. Quick vortex or vigorous shaking accelerates but is optional.
5. Under aseptic conditions, add 0.5 mL Buffer B to the Extralink vial. Invert several times to dissolve.
6. Within 4 hours of preparation, mix equal volumes of Glycosil and Gelin-S. For a 1.25 mL gel, combine 0.5 mL Glycosil + 0.5 mL Gelin-S. Mix by pipetting or gentle inversion.
7. Protect all dye/hydrogel mixtures from light.
8. For 1.25 mL hydrogel, add 0.025 mg dye to the Glycosil/Gelin mixture. Allow the reaction to proceed for 2 hours at room temperature.
9. (Optional, if encapsulating cells): Add 100 µL cell suspension to 2.0 mL Glycosil + Gelin-S. Mix gently by pipetting.
10. To initiate gelation, add Extralink to the Glycosil + Gelin-S mixture at a 1:4 volume ratio.
  - Example: 0.25 mL Extralink + 1.0 mL Glycosil/Gelin mixture → 1.25 mL hydrogel (1.35 mL total if cells are included).
  - Gelation begins within 10–15 minutes.