



## Fluorescent Labeling Hydrogels

This protocol describes how to label HyStem<sup>®</sup>-C hydrogels either blue or green using Alexa Fluor<sup>®</sup> 350 C5-maleimide or Alexa Fluor 488 C5-maleimide use the following protocol. Note that any fluorescent label can be used so long as it is a C5-maleimide that is thiol reactive. This protocol will make 1.25 mL of HyStem-C that will be labeled with Alexa Fluor 350 C5-maleimide.

### Required Materials

HyStem-C Hydrogels  
Alexa Fluor 350 C5-maleimide  
Alexa Fluor 488 C5-maleimide

### Notes

Thiol-reactive Alexa Fluor 350 C5-maleimide and its conjugates exhibit an intense, blue fluorescence. Alexa Fluor 350 protein conjugates are optimally excited at ~346 nm and emit at a wavelength slightly shorter than that of AMCA or AMCA-X conjugates (~442 nm versus ~448 nm). Thiol-reactive Alexa Fluor 488 C5 maleimide and its conjugates exhibit a bright, green fluorescence. Alexa Fluor 488 dye, which is spectrally similar to that of fluorescein, is pH-independent from pH 4 to 10.

### Procedure

1. Remove Glycosil<sup>®</sup>, Gelin-S<sup>®</sup>, and Extralink<sup>®</sup> vials from the -20 °C freezer and heat them to 37 °C (~30 minutes).
2. Remove the DG Water from the -20 °C freezer and thaw in a 37 °C water bath (~15 minutes).
3. Prepare a stock solution of Alexa Fluor 350 C5-maleimide in DG Water by adding 0.025 mg to 10 µL of water.

If you are using a different fluor, verify its solubility in water before proceeding. Make up only as much solution as you will use in one day since they are unstable and light sensitive. Read the manufacturer's guidelines for making the stock solution to determine if additional steps are required to properly prepare the solution. The optimal pH for reaction of maleimides with thiols is ~7.0.

4. Under aseptic conditions and using a syringe with the exact amount of liquid, add 1.0 mL of DG Water to the Glycosil vial. Repeat for the Gelin-S vial.
5. 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. Vigorous shaking will speed up dissolving time.
6. Under aseptic conditions and using a syringe with the exact amount of liquid, add 0.5 mL of DG Water to the Extralink vial. Invert several times to dissolve.



7. As soon as possible, but within four hours of making the solutions, mix equal volumes of Glycosil and Gelin-S. For a 1.25mL gel, combine 0.5mL Glycosil and 0.5mL Gelin-S. To mix, pipette back and forth or invert the vial.

8. Protect the dye and hydrogel reaction from light.

9. For 1.25 ml hydrogel, 0.025 mg dye will be needed.

10. Add dye to the Glycosil/Gelin mixture. Allow reaction to proceed for two hours at room temperature.

11. If encapsulating cells, add 100  $\mu$ L of cells to 2.0 mL of Glycosil + Gelin-S. Pipette back and forth to mix.

12. To form the hydrogel, add Extralink to the Glycosil + Gelin-S mix in a 1:4 volume ratio (0.25 mL Extralink to 1.0 mL Glycosil + Gelin-S). The total hydrogel volume with cells is 1.35 mL.

Note: Gelation will occur within ~20 minutes.