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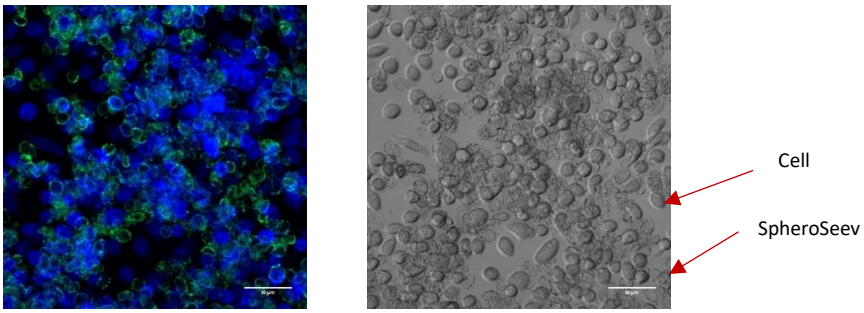
User Manual for Staining SpheroSeev and HydroSeev in Culture

Thioflavin S (Merck) is a fluorescent stain that changes the excitation and emission spectra when bound to beta-sheet rich structures to give a green color.
Thioflavin S stains beta-sheet rich SpheroSeev and HydroSeev biopolymer green (excitation 488nm, emission 530nm) and cells blue (excitation 405nm, emission 480nm).

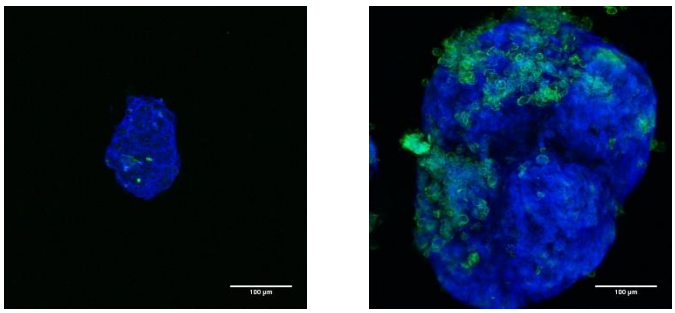
The following pictures demonstrate the use of Thioflavin S in a tissue culture of monolayer, spheroids and hydrogel.

I. L929 cells cultured on a tissue culture plate with SpheroSeev fibers stained with Thioflavin S. Green: SpheroSeev. Blue: L929 cells.



Merging of fluorescent channels Brightfield image

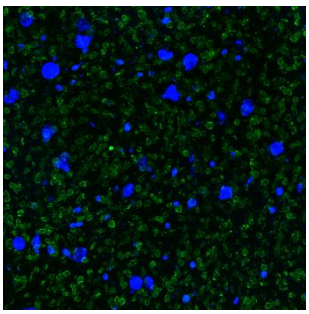
II. Mesenchymal stem cell spheroids seeded in ultra-low attachment plates with and without SpheroSeev. Green: SpheroSeev. Blue: L929 cells.



MSC control MSC spheroid with SpheroSeev

III. Alginate hydrogel printed with L929 cells and HydroSeev.

Green: HydroSeev. Blue: L929 cell aggregates.



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Thioflavin S preparation protocol

1. Dissolve 10 mg Thioflavin S (cat # T1892, Sigma) in 1 ml DMSO.

Spheroid staining protocol

1. Dilute Thioflavin S solution 400-fold in PBS (add 2.5µl thioflavin S to 975 µl PBS, 0.025 mg/ml final conc.).
2. Spheroids can either be collected into an Eppendorf tube (several spheroids can be stained in a single tube) or stained in the original multi-well plate.
3. Aspirate the medium from the wells or tube containing the spheroids. (Leave enough medium so as not to lose the spheroids themselves, ~30 µl medium/well in a 96-well plate or 100 µl medium/Eppendorf.)
4. Add the diluted dye (100 µl should be added to a well in a 96-well plate or 400-500 µl in an Eppendorf tube).
5. Incubate spheroids for 15 minutes at room temperature or 37°C. During the incubation step, floating spheroids will precipitate to the bottom of the tube. Otherwise, centrifuge tube for 2 min at 150 g.
6. Remove the thioflavin S from the well or tube (remove the same volume that was added in step 4).
7. Add PBS to each well or tube (100 µl/well of a 96-well plate or 500 µl/Eppendorf).
8. For high quality microscopy, it is recommended to transfer the spheroids into a black glass-bottom plate.
9. Observe culture under a fluorescent microscope.
   SpheroSeev: excitation 488nm, emission 530nm
   Cells: excitation 405nm, emission 480nm

Hydrogel staining protocol

1. Dilute Thioflavin S solution 4-fold in PBS (add 250µl thioflavin to 750 µl PBS, 2.5 mg/ml final concentration).
2. Aspirate the medium from the wells containing the model.
3. Wash the models with PBS (X1).
4. Add the diluted dye (cover the model).
5. Incubate cellular models for 60 minutes at room temperature or 37°C.
6. Remove the thioflavin S from the well.
7. Add PBS to each well (100 µl/well of a 96-well plate or 300 µl/well of a 48-well plate).

8. For high quality microscopy, it is recommended to transfer the model into a black glass-bottom plate.
9. Observe culture under a fluorescent microscope.
   HydroSeev: excitation 488nm, emission 530nm
   Cells: excitation 405nm, emission 480nm