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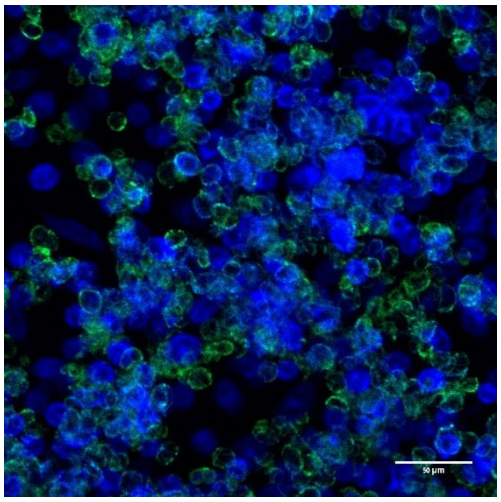
User Manual for Staining SpheroSeev in Culture

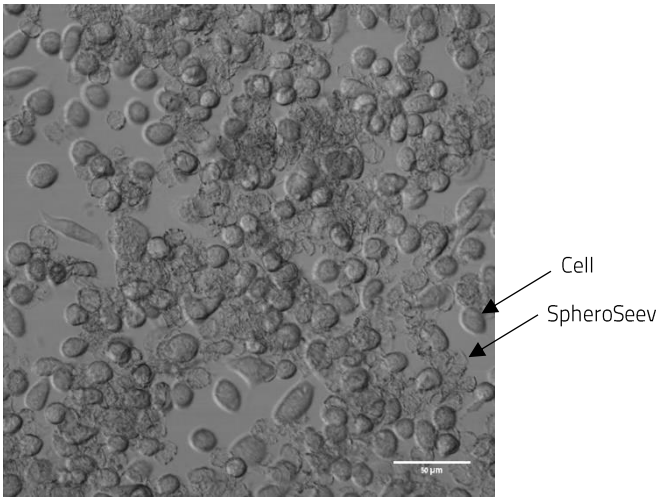
Thioflavin S (Merck) is a fluorescent stain that changes the excitation and emission spectra when bound to beta-sheet rich structures to give a green color.
SVX-Dye stains beta-sheet Reich SpheroSeev fibers green (excitation 488nm, emission 530nm) and cells blue (excitation 405nm, emission 480nm).

The following pictures demonstrate the use of SVX-Dye in a tissue culture of spheroids and monolayer.

I. L929 cell culture on a tissue culture plate with SpheroSeev fibers stained with SVX-Dye. Green: SpheroSeev fibers. Blue: L929 cells.



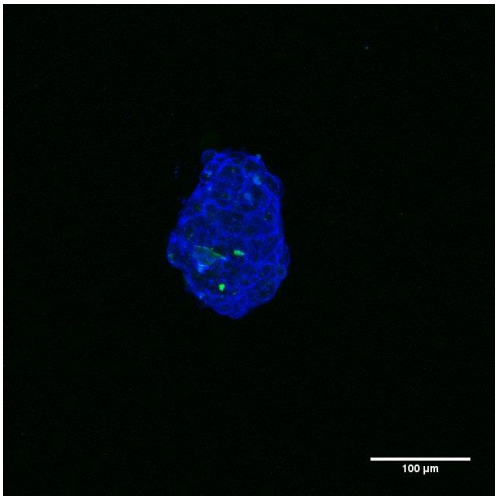
Merging of fluorescent channels



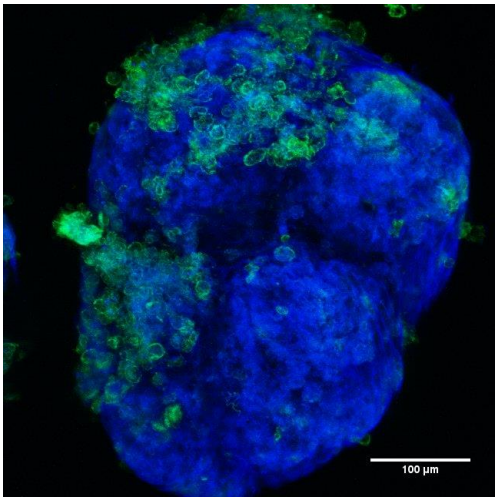
Brightfield image

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

MSC control



MSC spheroid with SpheroSeev



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Staining protocol

1. Dissolve 10 mg Thioflavin S (cat # T1892, Sigma) in 1 ml DMSO.
2. Dilute Thioflavin S solution 400-fold in PBS (add 25µl SVX-Dye to 975 µl PBS).
3. 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.
4. 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.)
5. 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).
6. 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.
7. Remove the SVX-Dye from the well or tube (remove the same volume that was added in step 4).
8. Add PBS to each well or tube (100 µl/well of a 96-well plate or 500 µl/Eppendorf).
9. For high quality microscopy, it is recommended to transfer the spheroids into a black glass-bottom plate.
10. Observe culture under a fluorescent microscope.
    SpheroSeev fibers: excitation 488nm, emission 530nm
    Cells: excitation 405nm, emission 480nm