

C. DESMIN STAINING

Set up culture in mini dish (35 x 10 mm) corning 430165, cell culture grade.
Allow to grow to 50-80% confluency. Note 1 T75 flask is equivalent to 5 of these plates.

Rinse 1x in PBS

Fix in 2% PFA for 1 min.

Wash 5x with PBS, 2 mins each wash.

Fix with 2% chilled acetone/PBS for 5 mins RT (pre-chill 15 mins at -20C)

Rinse 2x in PBS. Store under PBS at 4C for up to 2 days, (sodium azide can be used to store longer).

Incubate in primary antibody 1-2 hours at RT. Desmin antibody (sigma D1033, raised in mouse) is used at 1:400 in 0.5% BSA/PBS. Overlay 200-400ul into centre of dish and cover with a coverslip (cut plastic 60mm coverslips into squares). Do not press coverslip down.



Wash 3x in PBS.

Incubate in secondary antibody, 30 mins RT. Add DAPI at 1:20,000 (1:20 of Matt's working stock) to the secondary. Secondary will be anti mouse, with a fluorescent tag. After addition of secondary antibody, all subsequent steps should be carried out under dimmed lights. Cover with foil for incubations.

Rinse 3x in PBS

Aspirate PBS, add 2 drops of gel mount, cover with coverslip and aspirate around the outside of the coverslip to dry the remainder of the plate. Allow to dry in a dark place at RT overnight.

Cut side wall off plate and tape onto a microscope slide.

Store at -20C in the dark.