

LM Immuno (Triple labeling) protocol for adherent cells

Fix in Paraformaldehyde (4%) and Glutaraldehyde (0.1%) (30 min)

Wash 3x (5 min) in PBS

Incubate in 0.1% Triton X for 15-30 min

Wash in PBS for 5 min

Pre-incubate for 30 min with BSAC

Block with GBB3 for 2 hours

Rinse for couple seconds using PBS

Incubate with mixture of 1st and 2nd Primary (including 1:10 GBB3) overnight

(Next Day) wash 3x for 5 min with PBS

Incubate with mixture of Alexa 488 and 568 (including 1:10 GBB3) for 4 hours

Wash 3x for 5 min with PBS

Incubate in 10 μ M TOPRO3 in PBS for 15 min

Wash briefly (couple of seconds) in PBS

Mount in Prolong Gold at room temp without trapping bubbles

Seal coverslip to slide with nail polish (Top Coat/ Quick drying type)

Store in dark at 4 °C until analysis

Note: Perform second antibody and TOPRO3 incubations in dark. All steps should be performed at room temp.