

## **LM Immunolabeling (Triple labeling) protocol for adherent cells**

Fix in Paraformaldehyde (4%) and Glutaraldehyde (only if the samples require a better PM preservation and works good for EM samples-0.1%) (30 min, 1 h if the sample is a tissue)

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 (best for Immunogold/EM samples)

Block with GBB3 for 2 hours

Rinse for couple seconds using PBS

Incubate with mixture of 1<sup>st</sup> and 2<sup>nd</sup> 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 4h

Wash 3x for 5 min with PBS

Incubate in 10  $\mu$ 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.

**Call Shiv (217-333-1214) if you have any questions.**