

Cell Staining Protocol

1. Fixation-

Grow cells to 50-70% confluency. Fix cells onto plates or slides with ****FRESH**** 4% paraformaldehyde in PBS OR ice cold methanol for 5 minutes. Wash 3 times with PBS.

To Prepare FRESH 4% paraformaldehyde: "Dissolve EM grade paraformaldehyde in PBS in small bottle with stir bar (2g into 50 mLs). Add a few drops of NaOH and heat in hood (keep bottle cap loose) at 60°C to dissolve. Cool to room temperature and adjust pH to 7.4. Make fresh prior to use."

2. Permeablization-

Permeablize cells by exposure to 0.1% Triton X-100 in PBS for 5 minutes. Wash 1 time with 0.1% Triton-X 100 in PBS.

3. Primary Antibody-

Add primary antibody at proper dilutions (must test antibody first at several concentrations to know best dilution- see p.149 of Harlow and Lane) in PBS + 30 mg/ml BSA + 0.1% Triton X-100. Incubate for 90 minutes.

Wash 3 times consecutively with 0.1% Triton X-100 in PBS.

4. Secondary Antibody-

Add secondary antibody at proper dilutions in PBS + 30mg/ml BSA + 5% normal donkey or goat serum (depending on secondary antibody source) + 0.1% Triton X-100. Incubate for 60 minutes (room temperature) to overnight (4 degrees Celsius). Dilutions for molecular probes antibodies ~1:500.

Wash 3 times with 0.2% Triton X-100 in PBS ~ 5 minutes.

5. If DAPI stain wanted:

Stain with 1 μ g/ml DAPI in PBS for 1 minute. Wash 3 times with 0.1% Triton X-100 in PBS.

6. Wash samples 3 times with PBS to remove triton-X 100, add anti-fade reagent, coverslip and fingernail polish.