

Immunostaining Cells Notch 18G

Reagents:

4% paraformaldehyde (PFA)
Phosphate Buffered Saline (PBS)
Triton-X
Fetal Bovine Serum (FBS)
Antibodies of choice: 1°, 2°
(Primary: Notch18G primary, Secondary: Goat anti-rat TRITC)
DAPI

Protocol

Fix

Remove media
Rinse 2x with PBS
Add 1 ml 4% PFA (made fresh) per plate, 3 min @ RT
Rinse 3x with PBS

Permeabilize

Triton X (1:1000)	10 μ l
FBS (5%)	500 μ l
PBS	<u>9.5 ml</u>
	10 ml

Place 1 ml/plate, 10 min @ RT

Primary Stain

Rinse 3x 10 min ea. with PBS
PBS + 5% FBS + 1° Antibody diluted to appropriate concentration (for 18G: 1:250)
Place 1 ml/plate, leave O/N @ 4°C

Secondary Stain

Rinse 3x 10 min ea. with PBS
PBS + 5% FBS + 2° Antibody (for goat anti-rat TRITC 1:200)
Place 1 ml/plate, leave 1 hr @ RT

Rinse 2x 10 min each with PBS
PBS + DAPI (1:1000) 10 min @ RT
Rinse 3x with PBS