

Quick Protocol-MeOH Fix

- For cells grown on #1.5 (0.17mm thickness), 25mm coverslips in a 6-well plate.

Procedure	Temp. (°C)	Solution	Volume	Time
Rinse Cells	37	HBSS	2mL	Quick wash
Fix Cells	-20	100% MeOH	2mL	5-10 min
Wash	R.T.	PBS	2mL X 6	Quick washes (aspirate well)
Block	R.T.	PBS + 3% BSA	1mL	1 hour
Primary Antibody	R.T.	PBS + 1% BSA + 1 ^o Antibody	30µL - 100µL	1 hour
Wash	R.T.	PBS	2mL, 4-5X	Quick washes (3-5 min)
Secondary Antibody	R.T.	PBS + 1% BSA + 2 ^o Antibody	100µL - 1mL	1 hour
Hoescht Stain	R.T.	PBS + Hoescht (1: 12,000 dilution)	1-2 mL	2 min
Wash	R.T.	PBS	2mL, 4-5X	Quick washes (3-5 min)

Mounting

- Mount Coverslips on slides using an anti-fade agent according to manufactures directions.
 - E.g. for Dako let dry O/N at room temperature, then store at 4°C.
 - Do not use mounting media that has DNA dye in it.

Notes:

- For immune cells BSA can be substituted for serum of the host species of the secondary (e.g. for Donkey and mouse Cy3 secondary use 5% Donkey serum plus 1% BSA).
- The procedure can be stopped during blocking step: i.e. block O/N at 4°C.
- If staining for actin, fluorescent phalloidin can be incubated together with the secondary antibody.