Quick Protocol-PFA 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	37 to R.T.	2-4% PFA in rinse buffer	2mL	10-30 min
Wash	R.T.	PBS	2mL, 4X	Quick washes
Inactivate PFA	R.T.	PBS + 100mM glycine	2mL	10 min
Wash	R.T.	PBS	2mL, 2X	Quick washes
Permeablize Cells	R.T.	PBS + 1% BSA + 0.1% TX-100	2mL	20 min
Block	R.T.	PBS + 3% BSA	1mL	1 hour
Primary Antibody	R.T.	PBS + 1% BSA + 1° Antibody	30µL - 100µL	1 hour
Wash	R.T	PBS	2mL, 4-5X	Quick washes
Secondary Antibody	R.T.	PBS + 1% BSA + 2° Antibody	100µL - 1mL	1 hour
Hoescht Stain	R.T.	PBS + Hoescht (1: 12,000 dilution)	1 mL	2 min
Wash	R.T	PBS	2mL, 4-5X	Quick washes

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 after glycine inactivation (i.e. leave in PBS O/N at 4°C) or 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.