Eva Wegel 22/05/2012

Immunostaining for Structured Illumination Microscopy

Please note before you start:
A 22mm x 22mm coverslip mounted in the centre of the slide is the maximum area that can be imaged on the OMX.
Use High Precision coverslips (Marienfeld (distributed through Cellpath), 22x22 mm or smaller, no.1.5 (DO NOT USE ANYTHING ELSE), thickness 170 μm ± 5).
Clean coverslips for 30 min in diluted hydrochloric acid (4 mL HCL conc. in 250 mL H2O) in a rack on a shaker, rinse thoroughly in demin. water followed by 30 min. EtOH abs. on a shaker. Allow to dry.
If you are detecting microtubules please refer to the **Microtubule Immunostaining for SIM** protocol.

Stock solutions:
16% formaldehyde, MetOH free (ThermoScientific #28906)

10x PBS, pH7.4

10% Triton X-100
10% NP40, Igepal Ca-630 Sigma-Aldrich I3021-100mL, non-ionic detergent

Grow adherent cells on coverslips for the required time or allow non-adherent cells to spread on ConA coated coverslips for 2h.

Pre-extraction (optional):
5s in PBS, 0.1% NP40: Dunk coverslips in buffer and immediately transfer to fixative.

Fixative:
PBS, 2% formaldehyde for 20 min: 3 mL/well in 6-well plate
or
PBS, 4% formaldehyde for 20 min

1st wash:
10 min in PBS, 0.1% Triton X-100: Transfer coverslips to fresh 6-well plate with wash buffer.

2 washes in PBS, 5 min each: suck off used buffer and add fresh.

Immunostaining:

Blocking for 30 min in PBS, 0.1% Tween 20, 3% BSA, 200 μL/coverslip in humidified chamber

Primary antibody in PBS, 0.1% Tween 20, 3% BSA for 1 h at RT or o/n at 4°C, 200 μL/coverslip in humidified chamber

3 5min washes in PBS, 0.1% Tween 20

Secondary antibody in PBS, 0.1% Tween 20, 3% BSA for 1h at RT, 200 μL/coverslip in humidified chamber in the dark

3 5min washes in PBS, 0.1% Tween 20

Postfixation, optional:

Transfer coverslips to 4% Formaldehyde in PBS for 10min

3 5min washes in PBS, 0.1% Tween 20

5 min in 2μg/mL DAPI, 200 μL/coverslip in humidified chamber in the dark

5 min wash in demin. Water

Remove as much water as possible but don't dry the cells out. Either mount in 13 μL Prolong Gold, leave in drawer o/n and only seal with nail polish the following morning. Or mount in 13 μL Vectashield and seal with nail polish. Air bubbles are best prevented by placing a needle under one side of the coverslip and slowly withdrawing it.