Cell Immunostaining

Lucho Fuentealba 2008

- Grow cells on 2-well chamber slides (Lab-tek II chambers, Nalge Nunc international cat# 154461)

- Fix cells in fresh 4% paraformaldehyde in PBS for 15 min

- Permeabilize by treatment with 0.2% Triton X100 in D-PBS (Dubelcco's-PBS, Gibco cat#14190) for 10 min

- Optional step for antigen retrieval: incubate cells in 0.5% SDS in D-PBS for 5 min (J. M. Robinson, D. D. Vandré, *Histochem Cell Biol.* 116, 119 (2001)

- Block with blocking solution: 5% goat serum and 0.5% BSA in D-PBS (or alternative blocking solution: 2.5% non-fat milk in PBS-TX100 0.05%) for 1 hour

- Optional phosphatase treatment step: add 500 μl of 8000 U/ml bacteriophage λ-phosphatase (New England Biolabs cat # P0753S) per slide chamber and incubated for one hour at 30^{o}C

- Apply primary antibody overnight at 4°C. Primary antibodies are diluted in 1:4 blocking solution:D-PBS. Use a volume of 500 μl for each Lab-tek II chamber
- Wash antibodies 2x 15 minutes with D-PBS

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- Apply secondary antibody: Alexa 488-conjugated anti-rabbit (1:1000; Molecular Probes), Cy3-conjugated anti-rabbit (1:500; Jackson Labs), or Cy3-conjugated anti-mouse IgM or IgG (1:500; Jackson Labs) for 1 hour at room temperature

- Wash 3x 15 minutes in D-PBS

- Remove slide chambers with the provided slide separator

- Mount slides with Vectashield (Vector, cat # H-1200) containing DAPI stain to visualize DNA