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Technical Data Sheet

For research use only

*Not intended or approved for
diagnostic or therapeutic use.*

Antibody Protocol

Immunofluorescence Procedure for Staining Phosphoinositides (PIPs) in Cultured Cells

1. Fix cells by adding with an equal volume of 4% paraformaldehyde to cells in media for 20 minutes at room temperature.
2. Wash three times with TBS.
3. Permabilize the cells with 0.5% Saponin (Sigma # S-7900, or similar) at RT for 15 minutes.
4. Wash three times with TBS.
5. Block with 10% Goat Serum in TBS either overnight at 4 °C or 30 minutes at 37 °C.
6. Add Echelon mouse anti-PIP antibody diluted in TBS to the concentration suggested on the technical data sheet (use 200 µL per well in an 8-well chamber slide). Incubate for 60 minutes at 37 °C.
7. Wash 3 times with TBS-Goat Serum 1%.
8. Add 200 µL /well biotinylated goat anti-mouse IgM (1:2000) or IgG (1:5000) in TBS. Incubate for 30 minutes at 37 °C. (Recommended secondary antibody is from Jackson ImmunoResearch Labs # 115-065-075 for IgM or # 115-065-146 for IgG; or similar secondary antibody).
9. Wash three times with TBS-Goat Serum 1%.
10. Add Streptavidin-AlexaFluor 488 (Molecular Probes # S-11223) at a dilution of 1:2000 in TBS. Incubate 200 µl/well for 30 minutes at 37 °C.
11. Rinse thoroughly with nanopure or distilled H₂O.
12. Dry completely.
13. Seal with mounting media and coverslip. Store at 4 °C in the dark.
14. View with a confocal or fluorescence microscope.

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TDS Z-9100 Rev: 2 (12/07/05)