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NBP1-19447 Protocol

Immunocytochemistry/Immunofluorescence Protocol for 53BP1 Antibody (NBP1-19447)

Immunofluorescence Protocol

1. Cell Culture
1) Incubate cover glasses in 50% H2SO4 for 1 hour using a porcelain rack (Thomas Scientific).
2) Wash cover glasses for 30 min in running tap water.
3) Rinse with dH20.
4) Incubate cover glasses in 40 ug/ml poly-L-lysine (MW ~70-90kD) for 1 hour at room temperature.
5) Wash cover glasses for 1 hour in running tap water.
6) Rinse cover glasses 3 times 5 min each in dH2O.
7) Dry cover glasses on filter paper in a dust-free area.
8) Sterilize cover glasses inside the laminar flow chamber under UV light for at least 4 hours.
9) Detach cell from the plastic surface by incubating them in trypsin solution.
10) Resuspend detached cells in culture medium and transfer them to culture dishes with the cover glasses.
11) Culture cells up to semi-confluency.

2. Fixation
1) Fix in 3% paraformaldehyde, 0.02% glutaraldehyde in PBS for 15 min at room temperature.
2) Permeabilize by dipping cells for 10 seconds in 100% methanol (-20 degrees C).
3) Incubate cells for 3-times 10 min in 0.5 mg/ml NaBH4 in PBS, pH 8.0 to reduce aldehyde groups and then rinsed with PBS.

3. Staining
1) Drain off culture medium and rinse cover slips with PBS.
2) Drain off PBS with any of the above mentioned fixation methods.
3) Wash in PBS 3-times 5 min.
4) Permeabilize with 0.01% Triton X-100 in PBS for 30 sec (if needed).
5) Wash in PBS 3-times 5 min.
6) Incubate in 1% BSA, PBS pH 7.5 for 30 min to block unspecific binding of the antibodies. (alternative blocking solutions are: 1% gelatine, 1 % bovine or horse serum)
7) Incubate with primary antibody in 1% BSA, PBS pH 7.5 for 60 min (or over night at r.t. depending on antibody concentration and the accessibility of the antigen).
8) Wash with PBS pH 7.5, 3-times 10 min.
9) Incubate 2nd antibody in 1% BSA, PBS pH 7.5, 60 min at r.t.
10) Wash with PBS pH 7.5, 3-times 10 min.