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NB100-142 Protocol

Serum protocol for Mre11 Antibody (NB100-142)

[[URL:https://www.novusbio.com/products/mre11-antibody_nb100-142]][[Caption:Mre11 Antibody]]

Immunoprecipitation Procedure
1. For IP reactions, start with extract (whole cell or nuclear) from around 3 million cells prepared in 0.5-1 ml lysis buffer (100 mM NaCl, 10 mM Tris HCl, 5 mM EDTA, 0.5% nonidet p40).
2. Cells are resuspended in lysis buffer, then incubated with rotation about 15 min at 4 degrees C.
3. The lysate is then centrifuged 5 min at 14000g to remove insoluble material.
4. To cleared lysate, add 1-3 ul of antiserum and incubate on ice for 30 min.
5. Collect immune complexes on Protein A Sepharose by adding 25 ul of a 50% slurry, and incubate with rotation for 1 hour at 4 degrees C.
6. The complexes are pelleted gently (5000g for 5-10 sec.) then washed with 1 ml lysis buffer.
7. Repeat the wash 2 more times.
8. Analyze the immunoprecipitates by SDS PAGE. This antibody works well for IP reactions from both human and mouse cells. The intact complex is stable and can be immunoprecipitated in many common lysis buffers (up to 0.5 M NaCl).
Western Blot Procedure
1. Run 50 ug of protein on a 4-20% Tris-glycine mini-gel at 125V for 90 minutes.
2. Equilibrate gel, nitrocellulose membrane, Whatman paper, and blotting pads in transfer buffer for 15 minutes.
3. Transfer protein to the membrane at 25V for 90 minutes.
4. Allow membrane to air-dry.
5. Block membrane with 1XPBS/3% BSA for 1 hour at room temperature (23-27 degrees C).
6. Wash membrane twice, for 5 minutes each, with 1XPBS/0.05% Tween-20 (PBST).
7. Incubate membrane with 1:5000 dilution of NB100-142 (anti-hMre11), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
9. Incubate membrane with goat anti-rabbit IgG-HRP, diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
10. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
11. Detect cross-reacting proteins using Renaissance Chemiluminescence Reagent Plus kit from NEN Life Sciences.
NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.
Immunofluorescence Procedure
A 5beta in situ extraction method [10mM Pipes, pH 6.8 / 0.2% Triton X-100 / 100mM MgCl2 / 100mM sucrose/ 10mM EGTA Beta on ice] followed by 4% paraformaldehyde fixation of tissues works well for immunofluorescence of anti-hMre11 (NB 100-142).
Please see reference: Franchitto, A., Pichierri, P., Blooms syndrome protein is required for correct relocalization of RAD50/Mre11/nbs1 complex after replication fork arrest. J. of Cell Biology, DOI: 10 (2002)
Immunohistochemistry - FFPE sections
I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.
III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of primary antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.
NOTES:
Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
All steps in which Xylene is used should be performed in a fume hood.
For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).