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# NB100-142 Protocol

**Immunoprecipitation Protocol for Mre11 Antibody (NB100-142)**

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.