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NB100-177 Protocol

Western Blot Protocol for Rad51C Antibody (NB100-177)

Western Blot Protocol

1. Preparation of samples for loading ~50-80ug of sample containing laemmli loading dye (containing SDS) at 90 degrees Celsius for ~2 minutes.
2. Load sample onto a 10% Tris-HCL gel and run for ~30 minutes at 200V (or until dye front reaches bottom of gel).
3. Place gel in transfer buffer for 10 minutes (192mM Glycine, 25mM Tris-HCL, 20% Methanol). Pre-soak two pieces of Whatman paper and PVDF, as well.

NOTE: The PVDF should be soaked in CH3OH for ~ 1minute, rinsed in ddH20 and then placed in transfer buffer.

4. Transfer the protein from the gel to the membrane using a semi-dry transfer apparatus. Run for 20 minutes at 20V.
5. Block non-specific proteins with blocking buffer #1 (10mM Tris-HCL pH 8.0, 300mM NaCL, 0.025% Tween 20) for 10 minutes. Then continue blocking in blocking buffer #2 (buffer #1 + 15% nonfat dry milk) for an additional hour, gently rocking at room temperature (RT) or overnight at 4 degrees Celcius.
6. Dilute the primary antibody (anti-Rad51C, NB 100-177) in antibody dilution buffer (blocking buffer #1 + 2% milk).
7. Wash the membrane briefly with some blocking buffer #1 and then add your diluted primary antibody.
8. Incubate the primary for 1 hour at room temperature, gently rocking. Again this can be done overnight at 4 Celcius.
9. Wash 3X with blocking buffer #1 for 10 minutes, each, gently rocking.
10. Incubate the diluted secondary antibody (anti-mouse IgG conjugated to HRP), diluted in antibody dilution buffer, for 1 hour at room temperature, gently rocking.
11. Wash 2X with blocking buffer #1 for 10 minutes, each, gently rocking. Wash 1X with blocking buffer #1 for 30 minutes, gently rocking.
12. Develop membrane with your chemiluminescent substrate.

NOTE: NIH 3T3 and HEK 293 whole cell extracts and mouse embryonic fibroblast cells have been used as positive controls for this antibody.