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NB100-109 Protocol

Western Blot Protocol for MTH1 Antibody (NB100-109)

Procedure Guide for NB100-109 Anti-MTH1

Western Blot Procedure

1. Gels, Whatman, and membranes are soaked in electroblotting buffer (25 mM Tris-HCl; 193 mM glycine; 20% methanol) for 15 minutes prior to transferring.
2. Proteins separated on SDS-polyacrylamide gels, are transferred to 0.22 micron nitrocellulose sheets by electroblotting in a Transblot BioRad transfer apparatus in 25 mM Tris, 192 mM Glycine, 20% Methanol at 150 mA (70 V). The transfer is carried out for 1 hour at 4 degrees C.
3. Following protein transfer, the filter is blocked with Blotto [1X TBST (10X TBST = 1.5 M NaCl; 100 mM Tris-HCl, pH 8.0; 0.5% Tween 20; 2% NP-40; 0.2% SDS); 5% Carnation dried milk; 0.02% sodium azide] for 1 hour at room temperature on a rotator.
4. Dilute NB100-109 1:500 in Blotto and incubate with the filter at 4C overnight on a rotator.
5. Wash filter 3 times in 1X TBST (50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.5) for 10 minutes at 4 degrees C. Secondary antibody (peroxidase conjugated goat anti-rabbit, Boehringer-Mannheim) is incubated with the blot for 30 minutes at room temperature. Cross-reacting proteins are detected using the Chemiluminescence Western Blotting Kit from Boehringer-Mannheim.

NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.