

NB500-101 Protocol

Western Blot protocol for CENPF Antibody (NB500-101)

[[URL: https://www.novusbio.com/products/cenpf-antibody_nb500-101]][[Caption:CENPF Antibody]]
Western Blot Procedure

- 1) Resolve aliquots (30 mg) of total HeLa cell lysate on a 8.5% lo-crosslinker gel (110:1, acryl:bis) for efficient separation and transfer.
- 2) Transfer to nitrocellulose membranes in 20 mM Tris-HCL (pH 8.0)/150 mM glycine/20% (vol/vol) methanol. Transfer time for a 1 mm thick gel is 1 hour 15 minutes at 450mA.
- 3) Block membranes for 1 hour with 5% (vol/vol) nonfat dry milk/TBS-T (20 mM Tris-HCL, pH 7.6/ 137 mM NaCl/0.1% TWEEN 20)
- 4) Incubate membranes for 1 hour at room temperature (RT) in NB 500-101 diluted 1:3000 in nonfat dry milk/TBS-T.
- 5) Wash 3X30 minutes at RT with TBS-T.
- 6) Incubate membranes with alkaline phosphatase conjugated anti-rabbit IgG for 1 hour (RT).
- 7) Wash 3 x 30 minutes at RT with TBS-T
- 8) Develop with ECL reagents (Amersham) and autoradiography.