

NB110-57379 Protocol

Western Blot protocol for DNA Ligase IV Antibody (NB110-57379)

Western Blot protocol specific for DNA Ligase IV Antibody (NB110-57379):
https://www.novusbio.com/products/dna-ligase-iv-antibody_nb110-57379

To prepare total cell lysates, spin down cells and re-suspension the pellet in PBS to make the final concentration around 3×10^6 cells / ml. This is a convenient cell density for many cell lines, but adjustments may be necessary for cell types that differ substantially in size and protein content. Prepare cell extracts in appropriate non-reducing or reducing sample buffer. In some cases reducing agents may disrupt the conformation that is recognized by a monoclonal detection antibody. Mix the cell suspension with an equal volume of non-reducing 2X SDS gel sample buffer (6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) or reducing 2X SDS gel sample buffer [non-reducing buffer plus 20 mM dithiothreitol (DTT)]. Sonicate the cells to fragment the DNA using 8-10 bursts of 2-3 seconds each.

1. Load cell extracts and separate proteins on a 12% SDS-PAGE gel.
2. Transfer the separated proteins onto an Immobilon P membrane (Millipore) and incubate the membrane for 1 hour at room temperature or overnight at 2-8 C in Blocking Solution (1 X PBS, pH 7.4 containing 5% dry milk).
3. Wash the membrane at room temperature for 30 minutes with 5 changes of Wash Buffer (1X PBS with 0.1% NP40,).
4. Incubate the membrane for 2 hours at room temperature or overnight at 2-8 C in Blotting Buffer (1 X PBS, pH 7.4 containing 5% dry milk) containing NB110-57379 (1.25ug/ml).
5. Wash the membrane at room temperature for 30 minutes with 5 changes of Wash Buffer.
6. Incubate the membrane at room temperature for 1 hour in Blotting Buffer containing an HRP conjugated anti-Rabbit IgG secondary antibody, diluted 1: 50,000 - 100,000.
7. Wash the membrane at room temperature for 30 minutes with 5 changes of Wash Buffer.
8. Detect with chemiluminescence reagents.