PhosphoThreonine Western Blotting

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Abstract

This protocol is for pSerine and pThreonine western blotting (optimized for detecting phospho FMRP protein after IP)

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Protocol

Step 1.

Run 40% of IP eluate on 10% gel Tris-Glycine

REAGENTS
Anti-Phosphoserine Antibody AB1603 by Emd Millipore

Step 2.

Transfer protein to PVDF membrane for 1hr at RT with ice pack (100V)

Step 3.

Cut membrane just below 180kD (top MW marker on benchmark prestained ladder)

REAGENTS
Bovine Serum Albumin (IgG-Free, Protease-Free) 001-000-161 by Jackson Immunoresearch

Step 4.

Cut Membrane at 82kD (the blue band above the pink band using benchmark prestained ladder).

**Note, the GST-tagged protein is 115 kD. Cutting the membrane in the places will help reduce non-specific binding.

Also, running a ladder in the middle of the gel will help you cut straight across the membrane in the event that the gel is transferred at an angle relative to the membrane.

REAGENTS
Bovine Serum Albumin (IgG-Free, Protease-Free) 001-000-161 by Jackson Immunoresearch

Step 5.

Block membrane with 5% BSA (IgG and Protease-Free)/TBST for 2hrs at RT

REAGENTS
Bovine Serum Albumin (IgG-Free, Protease-Free) 001-000-161 by Jackson Immunoresearch

Step 6.

Probe membrane with phospho-threonine-HRP antibody in IgG-Free/Protease-Free BSA/TBST (1:1000). Probe overnight at 4C in cold room on tilting tray.

REAGENTS

Phospho-Threonine Antibody (P-Thr-Polyclonal) (HRP Conjugate) #6949 6949 by Cell Signaling Technology

Step 7.
Rinse membrane 8x over the course of 2hrs with 1x TBST

Step 8.
Activate membrane with pico ECL

**Note, the anti-pThr antibody is HRP conjugated so you don't need to use a secondary.

REAGENTS

- SuperSignal™ West Pico Chemiluminescent Substrate (Pico) 34080 by Contributed by users