

PhosphoSerine Western Blotting

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Abstract

This protocol is for pSerine and pThreonine western blotting

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Materials

Anti-Phosphoserine Antibody [AB1603](#) by [Emd Millipore](#)

SuperBlock T20 (PBS) Blocking Buffer [37516](#) by [Thermo Scientific](#)

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

Protocol

Step 1.

Run 40% of IP eluate on 10% gel



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 rabbit-anti-phosphoserine antibody from millipore in IgG-Free/Protease-Free BSA/TBST (1:2000, you can try 1:1000 if signal is not great at 1:2000). Probe overnight at 4C in cold room on tilting tray.

REAGENTS

Bovine Serum Albumin (IgG-Free, Protease-Free) [001-000-161](#) by [Jackson ImmunoResearch](#)

Step 7.

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

Step 8.

Block membrane in 5% milk/TBST for 1hr

Step 9.

Probe with mouse-anti-rabbit light-chain specific secondary antibody at 1:2000 for 2hrs at RT

REAGENTS

✓ Mouse Anti-Rabbit light chain, HRP conjugate Antibody [MAB201P](#) by Contributed by users

Step 10.

Rinse 6x with TBST over 1hr at RT

Step 11.

Activate membrane with pico ECL

REAGENTS

✓ SuperSignal™ West Pico Chemiluminescent Substrate (Pico) [34080](#) by Contributed by users

Step 12.

Visualize with Amersham film

REAGENTS

Amersham Hyperfilm MP [28-9068-46](#) by [Ge Life Sciences](#)