

# PhosphoTyrosine Western Blotting

**Christopher Bartley**

## Abstract

This protocol is for phospho-Tyrosine western blotting (optimized for detecting phospho FMRP protein after IP)

**Citation:** Christopher Bartley PhosphoTyrosine Western Blotting. **protocols.io**

dx.doi.org/10.17504/protocols.io.c47yzm

**Published:** 01 Nov 2016

## Protocol

### Step 1.

Run 80% of IP eluate on 10% Tris-Glycine 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-tyrosine antibody from millipore in IgG-Free/Protease-Free BSA/TBST (1:1000). Probe overnight at 4C in cold room on tilting tray.

#### REAGENTS

✓ Phospho-Tyrosine (P-Tyr-1000) MultiMab™ Rabbit mAb mix [8954](#) by Contributed by users

#### 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](#)

#### Step 13.

Strip with Restore Stripping buffer (10minutes at RT)

#### REAGENTS

✓ Restore™ Western Blot Stripping Buffer [21059](#) by Contributed by users

#### Step 14.

Rinse 1x with TBST

#### Step 15.

Block with 5% milk/TBST for 30 minutes

#### Step 16.

Probe with rabbit-anti-GST (1:2,000) overnight at 4C in cold room in 5% milk.

#### REAGENTS

✓ GST (91G1) Rabbit mAb [2625](#) by Contributed by users

#### Step 17.

Rinse 5x with TBST over 1hr

#### Step 18.

Probe with anti-rabbit at 1:2,000 in 5% milk/TBST for 1hr at RT

#### REAGENTS

✓ Anti-rabbit IgG, HRP-linked Antibody [7074](#) by Contributed by users

#### Step 19.

Rinse 5x over 1hr with 1x TBST at RT

**Step 20.**

Activate membrane with Pico ECL



**REAGENTS**



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

**Step 21.**

Visualize with Amersham Hyperfilm



**REAGENTS**

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