

# FMRP 1C3 Western Blot (Millipore MAB2160) post-IP

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## Abstract

Protocol for using Mouse-FMRP antibody follow immunoprecipitation of FMRP using a rabbit antibody such as Abcam #17722

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## Guidelines

This protocol is still in progress, will be updated periodically.

## Materials

Goat anti-Mouse light chain-specific antibody (HRP conjugated secondary) [115-035-174](#) by [Jackson ImmunoResearch](#)

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

Anti-Fragile X Mental Retardation Protein Antibody, clone 1C3 [MAB2160](#) by [Jackson ImmunoResearch](#)

## Protocol

### Step 1.

Run tris-glycine gel

### Step 2.

Transfer protein to PVDF

### Step 3.

Rinse membrane in 1x TBST

### Step 4.

Block in 5% non-fat milk/TBST for 30 minutes at RT

### Step 5.

Incubate membrane with anti-FMRP 1C3 at 1:4000 at RT for 1hr or O/N at 4C in 5% non-fat milk/1x TBST

### Step 6.

Rinse membrane 5x with 1x TBST over 30 minutes

### Step 7.

Block with 5% IgG-Free, Protease-Free Bovine Serum Albumin in 1x TBST for 30-45 minutes at RT

**Step 8.**

Add HRP-conjugated goat-anti-mouse IgG, light-chain specific antibody at 1:10,000

**Step 9.**

Incubate for 1hr at RT

**Step 10.**

Rinse 5x with 1x TBST over 30 minutes

**Step 11.**

Visualize with ECL solution according to manufacturer's protocol