

Incubate membrane in blocking buffer (1X TBST with 5% w/v nonfat dry milk (cell signaling technologies #9999) for 1 hr at room temperature.

Wash three times for 15 min each with TBST.

Incubate membrane and primary antibody in 10 ml primary antibody in 1X TBST with 5% w/v nonfat dry milk with gentle agitation overnight at 4°C.

Wash three times for 15 min each with TBST.

Incubate membrane with Anti-mouse IgG, Peroxidase-linked Antibody (401215-MerckMillipore, 1:8000 diluted) in blocking buffer with gentle agitation for 1 hr at room temperature.

Wash three times for 15 min each of TBST.

Place blot on a piece of plastic wrap on bench. Add Luminata Crescendo Western HRP substrate (WBLUR0100) on top of the blot for 3-5 min.

Wipe off excess solution from the blot and wrap securely in a plastic wrap.

Expose blot to x-ray film for 30 seconds -5 min.