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Cayman Chemical General Protocol for Western Blotting

Blocking: Immediately after proteins have been transferred to either nitrocellulose or PVDF membrane, place the membrane in 20 ml of blocking solution (0.5% non-fat dry milk in TBS pH 7.4). Incubate 1-2 hours at room temperature with gentle shaking or overnight at 4 degrees C (for long term storage of the blocked membrane: 3-4 days at 4°C addition of sodium azide to 0.02%, or alternatively freeze blot at -20°C wrapped in plastic/plastic bag).

Wash 1: Pour off the blocking solution and wash the membrane 3 times with TBS + 0.1% Tween-20 for 5 minutes per wash.

Primary Antibody Incubation: Primary antibody dilutions are carried out in 0.5% non-fat dry milk in TBS pH 7.4 plus 0.1% Tween-20 or a similar buffer. Membranes should be incubated in the primary antibody for at least 1 hour. Increased sensitivity may be achieved by incubating for longer periods of time (at room temp. or 16-18 hrs at 4 degrees C.)

Wash 2: Wash the membrane at least three times; same as wash 1.

Secondary Antibody Incubation: An enzyme linked anti-rabbit (for polyclonal antibodies) or anti-mouse (for monoclonal primary antibodies) secondary antibody is diluted according to the manufacturer specifications in blocking buffer. Incubate on the membrane with gentle shaking for 1 hour at room temp.

Wash 3: Wash the membrane at least 5 times (5 minutes/wash), same wash buffer as in wash 1 and 2.

Development: Develop the blot using either ECL or colorimetric technique according to the manufacturer specifications.

We currently recommend adapting the immuno-precipitation protocol listed in following guide: "Antibodies: A Laboratory Manual" (by Harlow and Lane 1988, Cold Spring Harbor Laboratory Press) to your cell/tissue lysate conditions.