

PrEST-BLOCKING PROTOCOL - IHC/ICC and WB

PrEST Antigens and the corresponding Triple A Polyclonals or PrecisA Monoclonals are recommended to use according to the standard PrEST-blocking protocol described below.

PrEST ANTIGENS IN PRE-ADSORPTION FOR IHC OR ICC EXPERIMENTS

Re-titrate the antibody in order to define the optimal dilution to be used in the blocking study. Optimal titration is the highest dilution possible at which antibody still shows expected staining.

Calculate 10x and 100x molar excess of the PrEST Antigen based on concentrations and MWs of antibody (150,000 Da) and PrEST Antigen (15,000 Da).

Mix calculated amounts of respective antibody and PrEST Antigen in 2ml tube and adjust total volume to 30 - 50 μ l with PBS.

Incubate tube containing antibody/PrEST Antigen for 30 min at room temperature, or 4°C overnight with gentle shaking.

As a control, use the primary antibody without PrEST Antigen, or a different PrEST Antigen (not corresponding to the target protein).

Centrifuge the tube with antibody/PrEST Antigen for 15 min at max speed (15,000 rpm) to pellet any immune complexes.

Carefully pipette the supernatant into a new tube to prepare a working solution for immunostaining. Since the antibody is now diluted 4 - 12 times, the volume of antibody diluent must be adjusted, accordingly.

Proceed with IHC experiment according to the general IHC, or ICC, protocol.

PrEST ANTIGENS IN PRE-ADSORPTION FOR WB EXPERIMENTS

Pre-incubate primary antibody diluted 1:250 with corresponding PrEST Antigen (100x molar excess) in 3,5 ml block buffer for 30 min at room temperature. 100x molar excess of PrEST Antigen is calculated based on concentrations and MWs of antibody (150,000 Da) and PrEST Antigen (15,000 Da).

As western blot control, include a primary antibody reaction without PrEST Antigen or use a different PrEST Antigen not corresponding to your antibody target.

Add incubated antibody-PrEST Antigen mixture to the blocked membrane and incubate on a roller mixer or rocking shaker for 1 hour at room temperature.

Proceed with WB experiment according to the general WB protocol.

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