

ANTIGEN BLOCKING PROTOCOL - IHC/ICC and WB

Antigen blocking protocol optimized for PrEST Antigens and the corresponding Triple A Polyclonals™ and PreciA Monoclonals™.

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 2 ml tube and adjust total volume to 30-50 µl 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 maximum 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 your 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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