Antigen Blocking Protocol for IHC/ICC and WB

This protocol describes antigen blocking optimized for Recombinant Protein Antigens and the corresponding antibody. Example would be catalog number NBP1-80586PEP (Recombinant Protein Antigen) and NBP1-80586 (antibody).

Pre-adsorption for IHC or ICC experiments

1. Re-titrate the antibody to define the optimal dilution used in the blocking study. Optimal titration is the highest dilution possible at which the antibody still shows the expected staining.
2. Calculate 10x and 100x molar excess of the Recombinant Protein Antigen based on concentrations and MWs of antibody (150,000 Da) and Recombinant Protein Antigen (15,000 Da). Mix calculated amounts of respective antibody and Recombinant Protein Antigen in a 2 ml tube and adjust the total volume to 30-50 µl with PBS.
3. Incubate tube containing antibody/Recombinant Protein Antigen for 30 min at room temperature, or +4°C overnight with gentle shaking.
4. As a control, use the primary antibody without the Recombinant Protein Antigen or a different Recombinant Protein Antigen (not corresponding to the target protein).
5. Centrifuge the tube with antibody/ Recombinant Protein Antigen for 15 min at maximum speed (15,000 rpm) to pellet any immune complexes.
6. 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.
7. Proceed with your experiment according to the IHC or ICC protocol for the corresponding antibody.

Pre-adsorption for WB experiments

1. Pre-incubate primary antibody diluted 1:250 with corresponding Recombinant Protein Antigen (100x molar excess) in 3,5 ml block buffer for 30 min at room temperature. 100x molar excess of Recombinant Protein Antigen is calculated based on concentrations and MWs of antibody (150,000 Da) and Recombinant Protein Antigen (15,000 Da).
2. As western blot control, include a primary antibody without Recombinant Protein Antigen or use a different Recombinant Protein Antigen not corresponding to your antibody target.
3. Add incubated antibody- Recombinant Protein Antigen mixture to the blocked membrane and incubate on a roller mixer or rocking shaker for 1 hour at room temperature.
4. Proceed with the WB experiment according to the WB protocol for the corresponding antibody.