

Tailor-made Antibodies and Tools for Life Science

WB: Antibody pre-Adsorption Protocol

Especially for polyclonal serum, this is a useful experiment to determine if an observed signal is related to the immunized antigen. If a signal disappears after pre-adsorption, the signal has a high probability of being specific. However, the possibility of cross-reactivity to other proteins sharing a similar antibody binding epitope cannot be excluded.

The pre-adsorption step can easily be implemented in a standard Western blot experiment. Carry out the pre-adsorption during the blocking step of your Western blot membrane.

Materials and reagents

- 5% skimmed milk in Tris buffered saline with Tween 20 (5% skimmed milk-TBST): 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% (w/v) skimmed milk, 0.1% Tween 20
- Primary antibody
- Blocking peptide/protein
- Two tubes
- Two identical blots

Procedure

- 1. Optimize antibody concentration in the appropriate buffer for your Western blot protocol.
- 2. Prepare the concentration-optimized antibody solution needed for two experiments.
- 3. Divide equally into two tubes.
- 4. Add 2-5 fold excess (by weight) of blocking peptide or protein to one tube. The final concentration can be optimized individually. This is the "blocked" or "pre-adsorbed" antibody solution.
- 5. Add an equivalent amount of buffer only to the other tube. This is the "control" antibody solution, which contains the same total volume as the "blocked" antibody solution.
- 6. Mix gently and incubate both tubes for 30-60 min at room temperature gently agitated.
- 7. Proceed with your normal Western blot protocol on the two identical blots, using the "blocked" antibody solution for one blot and the "control" antibody solution for the other.
- 8. Compare the "blocked" and "control" blots. The signals that are absent when using the "blocked" antibody are specific to the antibody.