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.