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.

Materials and solutions needed

- **5% skimmed milk-TBST (for westernblots) (**20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% (w/v) skimmed milk, 0.02% sodium azide, 0.1% Tween 20).
- **Incubation buffer (for ICC and IHC):** 5% normal serum, 0.1% Triton X100 in PBS; Triton may be omitted. Normal serum from the host-species of the secondary antibodies is recommended for blocking.
- Primary antibody
- Blocking peptide/protein
- Two tubes
- Two identical blots/slides/tissue sections

Procedure

1. Optimize antibody concentration in the appropriate buffer for your WB, ICC or IHC 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 staining protocol on the two sets of identical samples, using the "blocked" antibody solution for one set of samples and the "control" antibody solution for the other.
8. Compare the "blocked" and "control" staining. The signals that are absent when using the "blocked" antibody are specific to the antibody.