Blocking Peptide Competition Protocol

MATERIALS/REAGENTS/BUFFERS

- Blocking buffer (Trident Universal Protein Blocking Reagent (GTX30963); or 5% non-fat dry milk/PBST (for WB); or 5% normal animal serum in PBS (for IHC))
- Primary antibody
- Blocking peptide
- Two tubes
- Two identical blots/slides

PROTOCOL

1. Optimize antibody concentration in the appropriate blocking buffer for your WB or IHC protocol.
2. Prepare the concentration-optimized antibody solution to the final volume needed for two experiments.
3. Divide equally into two tubes.
4. In one tube, neutralize the antibody by adding 5- to 10-fold excess (by weight) blocking peptide. This is the "blocked" antibody solution.
5. In the other tube, add an equivalent amount of buffer only. This is the "control" antibody solution, which contains the same total volume as the "blocked" antibody solution.
6. Mix gently and incubate both tubes at room temperature for 30-60 minutes or overnight at 4°C.
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.

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