Peptide Competition Protocol

This protocol can be used to prepare "blocked" antibody for use in either western blotting or immunohistochemistry.

1. Determine the amount of antibody that is needed for your blocking experiment. For example, if your normal experiment is done at a 1:1000 dilution and you need 300ul to stain one slide you would need 0.3 ul of antibody in 299.7 ul of buffer, 300 ul for each slide.

2. Dilute the appropriate amount of antibody in 100 µl PBS/TBS. Make 2 tubes. To one tube add the blocking peptide solution at a 5-10:1 ratio to your antibody, i.e. if you added 1 ug of antibody, add 5-10 ug of blocking peptide. Bring the volume to 200ul with PBS/TBS. Add the same volume of saline/PBS (no peptide/antigen) to the antibody in the other tube and label as "no peptide". Mix gently.

3. Incubate both tubes at room temperature for 1hr or overnight at 4°C.

4. After incubating bring the volume of supernatant to 300 ul (or what is necessary depending upon the amount of antibody used initially) with buffer (PBS-Tween or your normal antibody staining buffer). Use both antibodies (with and without peptide) for staining using your normal staining protocol.

5. Compare staining patterns between the blocked and un-blocked antibodies.