BLOCKING WITH IMMUNIZING PEPTIDE (BL) PROTOCOL

Non-specific binding of an antibody to proteins other than the antigen can sometimes occur. This is usually more common with polyclonal antibodies, but can also occur with monoclonals as well.

To determine which band or staining is specific, an immunizing peptide blocking experiment can be performed. Before proceeding with the staining protocol, the antibody is neutralized (incubated with an excess of peptide that corresponds to the epitope recognized by the antibody). The antibody that is bound to the blocking peptide is no longer available to bind to the epitope present in the protein on the Western blot or in the cell. The neutralized antibody is then used side-by-side with the antibody alone, and the results are compared. By comparing the staining from the blocked antibody versus the antibody alone, you can see which staining is specific: this staining will be absent from the Western blot or immunostaining performed with the neutralized antibody.

Materials and Reagents

- Blocking buffer (usually TBST plus either 5% non-fat dry milk or 3% BSA for Western blot, or PBS plus 1% BSA for IHC)
- Antibody
- Blocking (immunizing) peptide
- Two tubes
- Two identical samples (e.g. a Western blot with two identical lanes, cut in half; two slides containing the cells of interest; etc)

Method

1. Determine the optimal concentration of antibody that consistently gives a positive result in your particular protocol. Using that concentration, determine how much antibody you will need for two experiments.
    a. For example, an antibody is being used successfully in Western blot at 0.5 µg/ml. You will need 2ml of antibody solution to stain one strip of a Western blot. Thus, you would use 1 µg of antibody in 2 ml buffer for each strip.
2. Dilute the necessary amount of antibody in blocking buffer to the final volume needed for the two experiments. Divide this equally into two tubes.
3. In the first tube, labeled ‘Blocked’, add the blocking peptide to a final concentration of 1 µg/ml (2 µg total peptide in this example).
In the second tube, labeled ‘Control’, add an equivalent amount of buffer.
4. Incubate both tubes, with agitation, at room temperature for 30 minutes, or overnight at 4°C.
5. Perform the staining protocol on the two identical samples, using the blocked antibody for one and the control for the other. Be careful not to mix up the strips using the blocked and control antibodies!

6. Observe the staining. The staining that disappears when using the blocked antibody is specific to the antibody. (See note i)

Notes

i. If more than one band disappears in Western blot by peptide/antigen competition, those bands contain the antigenic determinants and could be fragments of the full antigen or a complex containing the antigen.