

## Using Blocking Peptides as Controls in Western Blotting and IHC

To determine which band or staining is specific, an immunizing peptide blocking experiment can be performed. Before carrying out the staining of the immunoblot, 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 can no longer bind to the epitope present in the protein on the Western blot. 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. A similar principal can be employed for IHC using two slides.

### Buffers and Reagents:

- **Blocking buffer** of choice (usually TBST plus either 5% non-fat dry milk or 3% BSA for Western blot, or PBS plus 1% BSA for IHC)
- **Primary antibody:** See our selection of monoclonals at <http://www.fitzgerald-fii.com/monoclonal-antibodies.html> or polyclonals at <http://www.fitzgerald-fii.com/polyclonal-antibodies.html>
- **Secondary antibody:** See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.
- **Blocking peptide:** (corresponding to the epitope recognized by the antibody)
- Two identical samples
  - o Western blot - two identical lanes, cut in half
  - o IHC - two slides containing the cells/tissue of interest

### General Procedure:

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.  
For example, an antibody is being used successfully in Western blot at 0.5 µg/ml. You will need 2 ml 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. Label one tube '+ BP' and add peptide to a final concentration of 1 µg/ml (2 µg total peptide in this example).
4. Label the other tube '-BP' and add an equivalent amount of buffer.
5. Incubate both tubes, with agitation, at room temperature for 30 minutes, or overnight at 4°C.
6. Perform the staining protocol on the two identical samples, using the blocked antibody for one and the control for the other.
7. Observe the staining. The staining that disappears when using the blocked antibody is specific to the antibody. (If more than one band disappears in Western blot by peptide competition, those bands contain the antigenic determinants and could be fragments of the full antigen or a complex containing the antigen).