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Western Blot

Blocking with immunizing peptide

Biorbyt develops biochemicals, antibodies and immunoassays of the highest quality. For Western Blot, Biorbyt provides a wide range of matching antibodies blocking peptides.

Non-Specific Binding & Blocking Peptides

Non-Specific Binding

On Western blots, you might sometimes see non-specific binding of an antibody to proteins other than the antigen. This shows itself as multiple bands rather than the 1 or 2 bands you expect. In general, this is more common with polyclonal antibodies, but you sometimes see it with monoclonals. To work out which band or staining is specific to your protein, you can carry out an immunising peptide blocking experiment.

Blocking Peptides

Before you run the staining protocol, the antibody has to be neutralised by incubating it with an excess of peptide that corresponds to the epitope recognised by the antibody. These peptides are called blocking peptides. Blocking peptides are the peptides used to raise the original antibody,

particularly in the case of polyclonals. The antibody that is bound to the blocking peptide is no longer available to bind to its epitope in the protein on the Western blot or in the cell. The neutralised antibody is then used side-by-side with the original antibody alone, using the original protocol, and the results are compared. By comparing the staining from the blocked antibody to that of the original antibody alone, you can see which staining is specific. Staining patterns or bands from the blocked antibody will be absent from the Western blot or immunostaining performed with the neutralised antibody.

Blocking with immunizing peptide (BL) protocol from Biorbyt

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. 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)

Note

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.