Peptide blocking protocol for Western blotting (WB)

By Alomone Labs

What are blocking peptides?

Blocking peptides comprise the original peptide antigen used in immunization to raise a primary antibody. Consequently, they are sometimes referred to as immunizing peptides. We have also previously referred to them at Alomone Labs as 'negative control antigens'. They correspond to the epitope (specific amino-acid sequence recognized by an antibody) on a target protein. Blocking peptides can be used to compete or block antibody binding, and are therefore a valuable control for antibody specificity in Western blotting (WB) and immunostaining experiments.

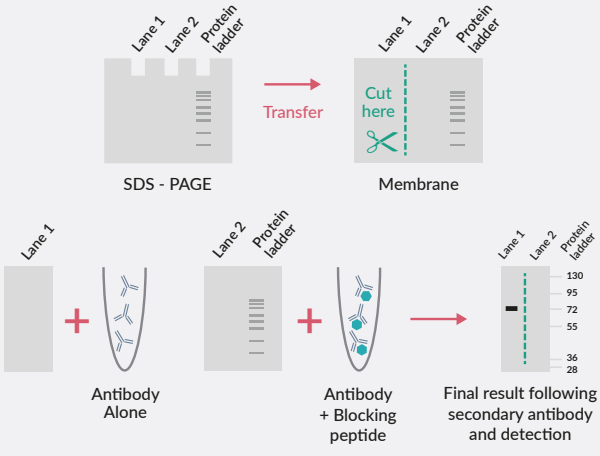
When are they used?

The inclusion of a blocking peptide as an experimental control is recommended, to help validate antibody specificity.

In a control sample, pre-incubation of an antibody with an excess of blocking peptide will occupy all the antibody binding sites. This prevents subsequent binding to the epitope in the protein of interest you are looking to detect.

A comparison of this staining (antibody + blocking peptide) versus a sample when you use the antibody alone, can help you determine non-specific binding. The staining that disappears when you use the antibody + blocking peptide, is specific to that antibody.

Schematic:
WB Peptide Blocking Protocol



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Peptide Blocking Protocol

Materials and Reagents

- Blocking buffer: Usually TBST (Tris-buffered saline, 0.1% Tween® 20) with either 5% non-fat dry milk, or 1% BSA + 0.05% NaN3
- Antibody blocking (immunizing) peptide
- Antibody
- Two tubes
- Two identical test samples. (Two lanes in WB with transfer membrane cut into strips to separate the samples for incubation)

Method

1. Reconstitute your lyophilized blocking peptide in sterile PBS or double distilled water (DDW) as per instructions (for example at 150 µg/ml). Ensure there is complete dissolution and triturate as necessary.

2. Optimize your WB protocol. Refer to our WB protocol for more information. Follow the product guidelines, or previous experimental optimizations for a recommended dilution for your primary antibody. Determine the quantity of antibody required for two experiments.

3. If your optimal antibody concentration is a 1:200 dilution, add 20 µg antibody to one 1.5 ml eppendorf tube, and 500 µl 1% BSA in PBS. Label it “antibody alone”

To a second identical tube, add 20 µg antibody, 20 µg negative control antigen (supplied with the antibody) and 500 µl 1% BSA in PBS. Label it “+peptide”.

4. Incubate both tubes for 1 hr at room temperature.

5. Transfer the content of each eppendorf to larger tubes and add 4.5 ml 1% BSA in PBS with 0.1% Tween® -20 and 0.05% NaN3 to each. Add the content of each tube to its respective test strip membrane for parallel experiments.

6. Incubate both tubes, with gentle rocking, overnight at 4°C.

7. Perform your WB staining protocol as normal on the two identical samples, using the antibody + blocking peptide (labeled "+ peptide") for one test strip as your primary antibody source, and the antibody alone for the other. Ensure you know which is which.

8. Proceed with thorough washes as per your WB protocol to remove unbound primary, and apply your secondary antibody for detection, repeat washing procedure.

9. Develop your blots, observe and compare the staining obtained in the two test strips. The band that disappears when using the blocked antibody is specific to the antibody. Other visible bands represent non-specific binding.

(NB: If multiple bands disappear, in addition to the one at the anticipated MW of your protein, it is important to consider the following:
If the MW is higher than expected - could your protein of interest have post-translational modifications?
If the MW is lower than expected - could your protein of interest have a truncated form, or could it have degraded in your sample?)

WB: Published customer data using Alomone Labs blocking peptide controls

Figure 1: WB using Anti-KISS1 Receptor (extracellular) Antibody (#AKR-001) with blocking peptide

Western blot analysis of human plasmacytoma (INA-6) cells, human mesenchymal stem cell (MSC), and human breast cancer cell lysates using Anti-KISS1 Receptor (extracellular) Antibody (#AKR-001), (upper panels). The band observed is completely eradicated when the antibody is preincubated with **KISS1 Receptor (extracellular) Blocking Peptide** (#BLP-KR001).

Adapted from Dotterweich, J. *et al.* (2016) *PLoS ONE* **11**, e0155087 with permission of PLoS.

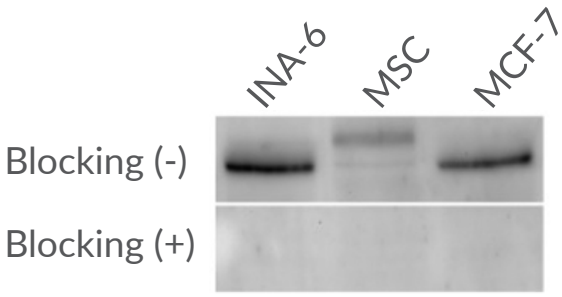

Figure 2: WB using Anti-P2X7 Receptor Antibody (# APR-004) with blocking peptide

Western blots show a P2X7R band (left panels) in epileptic mouse and human hippocampal samples (n = 1/lane) and elimination of the band (middle panels) when Anti-P2X7 Receptor Antibody (# APR-004) was preincubated with **P2X7 Receptor Blocking Peptide** (#BLP-PR004) (+pep7r).

Right panels show the band remains when antibodies were preincubated with the unrelated **P2X4 Receptor Blocking Peptide** (#BLP-PR004) (+pep4r).

Adapted from Jimenez-Pacheco, A. *et al.* (2016) *J. Neurosci.* **36**, 5920 with permission of the Society for Neuroscience.