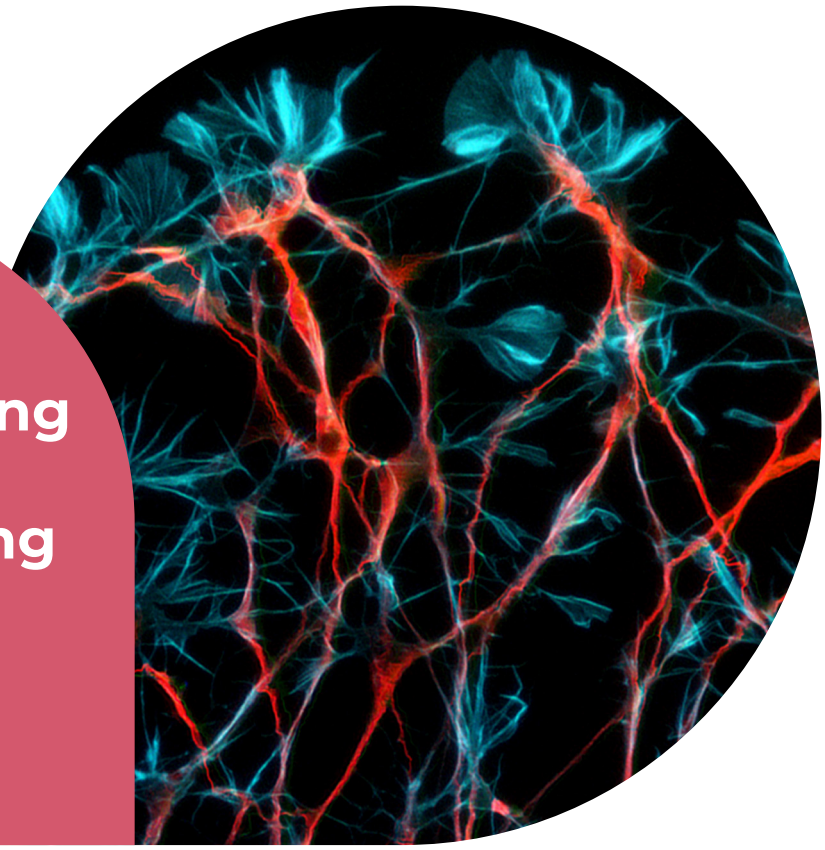


Peptide blocking protocol for immunostaining (IHC, ICC, IF)

By Alomone Labs

What are blocking peptides?

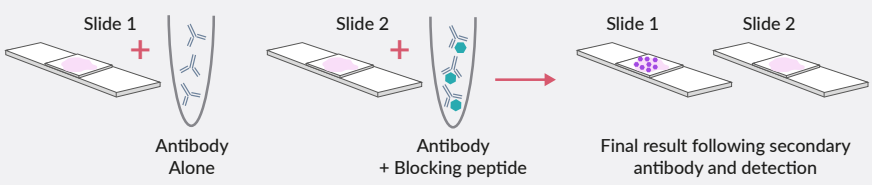
Blocking peptides comprise the original peptide antigen used in immunization to raise a primary antibody. Consequently, they are sometimes referred to as immunizing peptides. We have also previously referred to them at Alomone Labs as 'negative control antigens'. They correspond to the epitope (specific 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 immunohistochemistry (IHC), immunocytochemistry (ICC) and immunofluorescent staining (IF) experiments. Note that they are not suitable for use as a control for applications requiring live and intact cells (flow cytometry, live cell imaging).

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 a primary 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:
Immunostaining Peptide Blocking Protocol



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Peptide Blocking Protocol

Materials and Reagents

- Antibody dilution buffer: Usually PBST (Phosphate buffered saline + 0.1% Tween 20) with 1% BSA
- Blocking (immunizing) peptide
- Antibody
- Two tubes
- Two identical test samples (Two identical slides containing the tissue or cells of interest)

Method

1. Complete the slide preparation, fixation and permeabilization (for intracellular antigens) of your tissue or cells as per your immunostaining protocol. Refer to our IHC protocol or ICC/IF protocols for further information. Proceed to the stage below, where your primary antibody is prepared, with or without pre-absorption with blocking peptide.

2. 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.

3. Optimize your IHC or ICC/IF protocol in advance. 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 (e.g If your optimal antibody concentration is at 2 µg/ml, you will require 2 µg of antibody if you are using 1 ml buffer, for each of your slides).

4. Dilute the necessary amount of antibody in dilution buffer to the final volume needed for the two experiments (4 µg in 2 ml in this example).

5. Divide this equally into two tubes.

6. Label the first tube "+peptide". Add ten times excess of blocking peptide to antibody (e.g If your peptide was at a concentration of 150 µg/ml, you would need to add 133 µl to give 20 µg total peptide in 1 ml buffer in this example).

7. In the second tube, labeled "antibody alone", add an equivalent amount of buffer volume (133 µl in this example).

8. Incubate both tubes, with agitation overnight at 4°C, or alternatively at room temperature for 30 min.

9. Perform your IHC or ICC/IF antibody staining protocol on the two identical sample slides, using the antibody + blocking peptide (labeled "+ peptide") for one slide as your primary antibody source, and the antibody alone for the other. Ensure you know which is which by labeling the slides.

10. Proceed with PBS washes as per your protocol to remove unbound primary, and apply the secondary antibody for detection, repeat washing procedure.

11. Mount your slides as per your protocol.

12. Observe and compare the staining pattern obtained in the two test slides. The staining that disappears when using the blocked antibody is specific to the antibody. Other staining that is visible represents non-specific binding.

IHC/ICC/IF: Published customer data using Alomone Labs blocking peptide

Figure 1: ICC using Anti-TRPV4 Antibody (#ACC-034) with blocking peptide

Immunocytochemical staining of mouse mCCDcl1 kidney cells using Anti-TRPV4 Antibody (#ACC-034), (green). TRPV4 staining is completely abolished when the antibody is preincubated with the TRPV4 Blocking Peptide (#BLP-CC034) (+ BP in right panel).

Adapted from Li, Y. *et al.* (2016) *PLoS ONE* **11**, e0155006 with permission of PLoS.

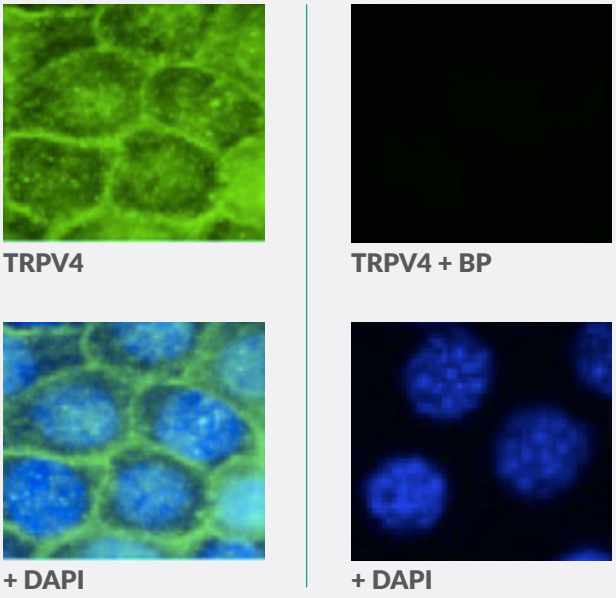

Figure 2: IHC using Anti-$Ca_V2.3$ Antibody (#ACC-006) with blocking peptide

Immunohistochemical staining of mouse accessory olfactory bulb sections using Anti-$Ca_V2.3$ (CAC-NA1E) Antibody (#ACC-006). A(i). $Ca_V2.3$ staining (green) is detected in mitral cells. A(ii). High-magnification of (i). A(iii). Note the absence of immunofluorescent staining when the antibody is preincubated with $Ca_V2.3$/CACNA1E Blocking Peptide (#BLP-CC006).

Adapted from Gorin, M. *et al.* (2016) *J. Neurosci.* **36,** 3127 with permission of the Society for Neuroscience.