



ABCEPTA CUSTOM SERVICES:

Blocking Peptide Protocol

We describe below general recommendations for using blocking peptides in western blot and immunostaining techniques. The precise conditions should be optimized for a particular assay.

Protocol:

Peptides are used to block antibody binding to its target. In order to visualize the inhibitory effect of the peptide, they are usually used at 10 × to 100 × excess compared to antibody molarity. We recommend using a 1 ×, 10 × and 100 × excess of peptide first, and then to narrow this range if a more accurate study is required.

Calculation

Abcepta antibodies are manufactured at 0.25 mg/ml. Using the antibody at 1:100 dilution as recommended corresponds to 2.5 ug/ml. Estimating the MW of an antibody at 150,000 Da, the final antibody concentration is ca. 17 nM. A peptide of 15 residues long has an average MW of 1650 Da (110 Da multiplied by 15).

For an excess of 100 × of peptide over the antibody used at 17 nM, a concentration of 1.7 uM or $1.7 \text{ uM} \times 1650 = 2.8 \text{ ug/ml}$, is needed. Since one antibody binds two peptides, 5.6 ug/ml is 100 × excess.

Important Note

It is very important to mix the antibody with the peptide before incubation with the cell lysate or onto slide. Otherwise, you may not be able to disrupt antibody binding from native target.