LP104 – BLOCKING PEPTIDES

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 10X to 100X excess compared to antibody molarity. We recommend using a 1X, 10X and 100X excess of peptide first, and then to narrow this range if a more accurate study is required.

Calculation

Abbiotec antibodies are manufactured at 1 mg/ml. Using the antibody at 1:200 dilution as recommended corresponds to 5 μg/ml. Estimating the MW of an antibody at 150,000 Da, the final antibody concentration is ca. 34 nM.
A peptide of 15 residues long has an average MW of 1650 Da (110 Da multiplied by 15). For an excess of 100X of peptide over the antibody used at 34 nM, a concentration of 3.4 μM or 3.4 μM x 1650 = 5.6 μg/ml, is needed. Since one antibody binds two peptides, 11.2 μg/ml is 100X 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.