Absorption Controls Using Blocking Peptides

One type of specificity control for anti-peptide antibodies is to pre-absorb the antibody with the same peptide (not conjugated to carrier molecule) used to immunize the animals that produced the antibody. This is often referred to as an *Absorption Control* or *Specificity Control* (Figure 1). The peptide is often refered to the *Blocking Peptide* or *Control Peptide*.

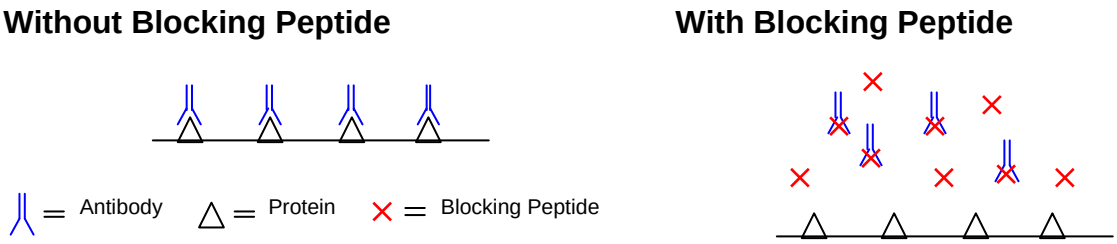

Figure 1. When performing an Absorption Control, you are performing a Competitive Assay. You are using the peptide to compete with the protein of interest in your sample for antibody binding. Therefore, you want to use the peptide in excess to favor the antibody binding with the peptide and inhibit binding with the protein of interest. Theoretically, any binding of the antibody to your sample in the presence of the peptide is non-specific.

Basic Protocol for Absorption Control

1) Make 2 vials of your primary antibody at the working concentration specified on the data sheet or at the best concentration determined by you empirically. 1 vial is for normally processing with your primary antibody, the other vial is for use with the Blocking Peptide.

2) Add the Blocking Peptide at the recommended dilution (see below) into 1 of the vials and incubate at room temperature for 1 hour.

3) Process your tissue/samples as normal with both antibody solutions.

Quick Calculation for Absorption Controls

When we perform Absoption Controls, we use the peptide at a final concentration of ~10uM (1 x 10^{-5}) or 1uM (1 x 10^{-6} M). Because most peptides are between 15-20 amino acids with a molecular weight of ~2000 Da, we supply most of our peptide at a concentration of 2mgs/ml. This allows for a simple dilution of 1:100 (~10 uM) or 1:1000 (~1uM) in the working antibody solution. If the antibody is supplied lyophilized, re-constitute it in dH_2O at a concentration of 2mgs/ml stock solution.