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# Using Control Antigen

## Using a Control Antigen for Negative Control

1. Dissolve a control antigen. If the control antigen is a peptide, dissolve it in 100µl distilled water. If it is a fusion protein, dissolve it in PBS, if not mentioned otherwise in the antibody datasheet.
2. After dissolving a fusion protein, keep it on ice. For longer periods, store it frozen, preferably at -70 ºC. A peptide can be stored at -20 ºC.
3. Dilute the necessary amount of an antibody in 200-500 µl PBS, containing 1% BSA and 0.025% $NaN_3$ (Tube 1). In parallel, dilute the same amount of the antibody in another vial (Tube 2).
4. Add to tube 2 the appropriate amount of a control antigen.
5. Incubate at room temperature for 1 h.
6. Centrifuge both tubes at 10000xg 5 min.
7. Use the supernatants from tubes 1 and 2 for parallel experiments.

## Using a Control Fusion Protein Antigen as a Positive Control in Western Blotting

*Notes.*
*In many cases, the control antigens that we supply are small peptides with Mw ~ 2000 Da. Such peptides cannot be used as positive controls in standard Western blotting.*

1. Dissolve a control fusion protein in 100 µl PBS, if not mentioned otherwise in the antibody datasheet. The concentration is now 0.5 mg/ml.
2. After dissolving a fusion protein, keep it on ice. For longer periods, store it frozen, preferably at -70 ºC. A peptide can be stored at -20 ºC.
3. Dissolve 1µl of a fusion protein in 100µl sample buffer (the concentration now is 5 µg/ml).
4. Apply 5-10 µl (25-50 ng) for a gel line.
5. Run a Western blotting experiment according to protocol 5.