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861-0005 Protocol

Antibody Concentration and Clean Up Protocol

1. INTRODUCTION

Antibodies are sometimes only available at low concentrations and often contain low molecular weight substances that interfere in labeling reactions with enzymes, biotin, streptavidin and fluorophores. The Antibody Concentration and Clean Up Kit allows for the quick and easy concentration of antibodies and proteins. The kit can also be used to reduce the concentration of many unwanted additives often found in antibody formulations such as azide, glycine or tris. The antibody clean up kit method utilizes a simple spin column to easily and quickly remove excess buffer from the antibody thereby providing a more concentrated antibody solution. The clean up kit also allows the experimenter to perform a simple buffer exchange to transfer the antibody into a more favorable conjugation buffer.

2. INSTRUCTIONS

2.1 Storage and components
The kit is shipped at ambient temperature. Store the kit at 4 degrees Celsius upon receipt.

2.2. Kit contents:
1 or 3 spin cartridge/collecting tube assembles
1 bottle of Conjugation Buffer

2.3 Concentration of Antibody Solution

Step 1: Add antibody to spin cartridge.

Step 2: Spin for 1 to 3 minutes* in a microfuge at maximum speed to reduce the buffer volume in the spin cartridge to between 50 and 100ul.

Step 3: Repeat steps 1 to 2 as many times as is necessary to process the entire antibody to the desired concentration. It may be necessary to discard the excess buffer collected in the collection tube between spins.

Step 4: Recover the concentrated antibody from the spin cartridge.

Note: We advise not spinnning the antibody dry as reconstitution of the antibody will be difficult, and significant antibody loss and degradation may occur.

*Spin times will vary depending on buffer composition and volume as well as centrifuge speed.

Other proteins present in the buffer such as BSA will also be concentrated using this method. To remove unwanted proteins see our Abselect kit described in section 4.

2.4 Buffer exchange using Spin Column assembly

Step 1: Add up to 0.5ml antibody to spin cartridge.

Step 2: Spin for 1 to 3 minutes* in a microfuge at maximum speed to reduce the buffer volume to 100ul.

Step 3: Discard the excess liquid in collection tube

Step 4: Add 400ul conjugation buffer to the antibody in the spin cartridge.

Step 5: Spin for 1 to 3 minutes* in a microfuge at maximum speed to reduce buffer volume to 100ul.

Step 6: Discard the excess liquid in collection tube

Step 7: Repeat steps 1 to 6 at least 5 times to exchange antibody buffer.

Step 8: Recover antibody from the spin cartridge.

Note. Each cycle leads to a reduction in the concentration of low molecular substances. By performing as many as 5 repeat steps the concentration of small molecules such as glycine and Tris will be reduced 2500 fold. However, the concentration of proteins such as BSA will be unchanged. To remove unwanted proteins see our Abselect kit described in section 4 The exchange process is more efficient if the volume is reduced to 50ul instead of 100ul at each cycle. *Spin times will vary depending on buffer composition and volume as well as centrifuge speed.

2.5 Storage of antibody Store at 4 degrees Celsius. Other storage conditions (e.g. frozen at -70 degrees Celsius may also be satisfactory). The sensitivity of any particular antibody to freeze thaw should be determined by experimentation on small aliquots.

3. TEST FOR PROTEIN

Wherever possible, protein values should be determined using an absorbance at 280nm. For an IgG using a 1cm light path an OD280 of 1.0 is equal to an antibody concentration 0.714mg/ml. When using Bradford type reagents it is important to use an IgG standard curve. The absorbance generated by this type of reagent is dependent on the protein used. For example using a BSA standard curve to determine the protein concentration of an IgG solution will result in a two fold under estimate of the IgG concentration.