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860-0010 Protocol

Purification Protocol (860-0010)

1. INTRODUCTION

Commercially available antibodies often contain substances (e.g. BSA, glycine, tris, azide) that interfere in labeling reactions with enzymes or fluorophores. The AbSelect Purification Kit quickly removes these contaminants; it can also be used to purify antibodies from crude samples such as ascites fluid or immune serum. The antibody to be purified or cleaned up ideally is in a volume of 100ul to 0.5ml. Up to 500ug of antibody can be purified in each run.

The AbSelect method involves capture of the antibody on protein A resin and the removal of unwanted substances by a simple wash procedure, which is carried out in a standard microfuge. The purified product is then eluted and neutralized.

The components of the AbSelect kit are fully compatible with the Lightning-Link conjugation system (available separately), which allows the purified antibody to be immediately labeled with HRP, AP or RPE with a hands-on time of under 30 seconds (see Related Products). Note: The AbSelect protein A/spin method cannot easily be used with samples containing relatively dilute antibody in large volumes (e.g. tissue culture supernatant). A protein A/gravity feed column method is more convenient in this situation (see Related Products).

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:

Lyophilised protein A resin
1 vial of 10x Binding Buffer
1 vial of Wash Buffer
1 vial of Elution Buffer
1 vial of Neutraliser
1 spin cartridge/collecting tube assembly
4 additional collecting tubes
Not supplied: BioRad protein reagent

2.3. Overview of procedure

Step 1 Reconstitute protein A resin with wash buffer
Step 2 Transfer to spin cartridge and spin briefly
Step 3. Add antibody supplemented with 10x binding buffer.
Step 4 Incubate for 1h and then wash beads
Step 5 Elute resin and neutralize purified antibody
Step 6 Confirm successful elution using spot test for protein.

2.4. Reconstution of protein A resin

Add 0.5ml of wash buffer, mix by inversion for a few seconds and transfer to the spin cartridge. Spin for 30 seconds in a microfuge.

2.5. Incubation of sample with resin

To the antibody, add an appropriate amount of 10x Binding Buffer. For example, if the sample volume is 200ul, add 20ul of Binding Buffer. Pipette the sample into the spin cartridge and cap the tube. Incubate for 1 hour with agitation, endover-end mixing or periodic shaking.

Note: The volume of antibody to be purified or cleaned up ideally should be 0.1-0.5ml, though larger volumes may be processed by first incubating the antibody sample with the protein A resin in a larger vessel (e.g. 2ml eppendorf) prior to transferring to the spin cartridge.

2.6. Wash procedure

Microfuge the spin cartridge assembly for 30 seconds to remove most of the non-bound protein. Add 0.5ml of wash buffer and spin again. Repeat the wash procedure three times. Note: Save the non-bound and wash fractions by transferring the material from the collecting tube after each spin to a set of eppendorfs (not supplied). Do not use the four collecting tubes supplied with the kit, as these have an extended hinge to accommodate the spin cartridge, and are required for the elution step.

2.7. Elution

Transfer the cartridge to a clean collecting tube. Add 100ul of elution buffer and incubate for 2 min at room temperature with gentle agitation. Microfuge for 30 seconds. Remove the collecting tube and add 25ul neutralizer to the tube.

Place the cartridge in a new collecting tube and add a further 100ul of elution buffer to the protein A resin. Incubate for 2 min at room temperature with gentle agitation. Spin and collect and neutralize as before.

Repeat the elution procedure until all four clean collecting cups have been used. The protein normally elutes in tubes 1 and 2 but you should confirm this using a test for protein (see note 3) before pooling any of the tubes. Pool the tubes with most protein (normally two tubes; if more than two tubes are strongly positive it is possible that you have used too much sample in your protein assay). However, if your application does not require a high concentration of antibody you may choose to pool all tubes that contain protein, regardless of concentration.

2.8. 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. When other methods of are used such as BCA or Bradford protein assays, determinations should be performed before the addition of the neutralization buffer. The neutralization buffer contains components that can interfere with these reagents. The neutralization buffer should be added to the sample as soon as possible as the low pH of the elution buffer can denature the antibody.

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.

4. Related Products

851-0024 Protein Agarose
701-0010 Horseradish peroxidase 30-second conjugation kit
703 -0005 R-Phycoerythrin 30-second conjugation kit