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AbSelect Mouse Antibody Purification System (1 Purification)

Catalog No.: AC052-001

Quantity: 1 set

Reaction: 1 Purification

Kit Contents:

AC052-001-R:	1 vial of AbSelect Mouse Resin
AC052-001-B:	1 vial of 10x Binding Buffer
AC052-001-W:	1 vial of Wash Buffer
AC052-001-E:	1 vial of Elution Buffer
AC052-001-N:	1 vial of Neutralization Buffer
AC052-001-T1:	1 spin cartridge / collecting tube assemblies

Background:

Commercially available antibodies often contain substances (e.g. BSA, glycine, tris and azide) that interfere in labeling reactions with enzymes, biotin, streptavidin or fluorophores. The AbSelect Mouse Antibody Purification System **AC052** quickly removes these contaminants. It can also be used to purify mouse antibodies from crude samples such as ascites fluid.

The Mouse antibody to be purified or cleaned up is in a volume of 0.1 ml to 0.5 ml. Up to 150 µg of antibody can be purified in each run. The method involves capturing the Mouse antibody on AbSelect Mouse Resin. The Resin has a high affinity for Mouse IgG molecules. Once the Mouse antibody has bound to the AbSelect Mouse Resin, unwanted substances can be removed by simply washing the resin. The purified product is then eluted and neutralized. The AbSelect Mouse Antibody Purification System **AC052** is not suitable for use with antibodies from other species. It should be noted that the binding strength of Bovine IgG to the AbSelect Mouse Resin is negligible.
The components of the AbSelect kit are fully compatible with the Link-A-Light antibody labeling system (available separately), which allows the purified antibody to be immediately labeled with a hands-on time of under 30 seconds.

Principle of the Procedure:

Step 1: Transfer AbSelect Mouse Resin **AC052-001-R** to the spin cartridge and spin briefly. **Step 2:** Add antibody supplemented with 10x Binding Buffer **AC052-001-B**. **Step 4:** Incubate for 1h and then Wash Resin. **Step 5:** Elute and neutralize purified Mouse antibody. **Step 6:** Confirm antibody is in eluate using a test for protein.

Protocols:

1. Reconstitution of AbSelect Mouse Resin
Add 0.3ml of Wash Buffer **AC052-001-W** to each vial of Abselect Mouse Resin, mix by inversion for a few seconds and transfer to the Spin cartridge **AC052-001-T1** (Figure 1). Spin for 30 seconds in a microfuge.

2. Incubation of Sample with Resin

Add the appropriate amount of 10x Binding Buffer **AC052-001-B** to the antibody. For example, if the sample volume is 200 µl, add 20 µl of Binding Buffer. Pipette the sample into the spin cartridge and cap the tube. Incubate for 1 hour with agitation or periodic shaking.

Note: The volume of antibody to be purified or cleaned up should be 0.1-0.5 ml, though larger volumes may be processed by first incubating the antibody sample with the Protein A Resin **AC052-001-R** in a larger vessel (e.g. 2ml eppendorf) prior to transferring to the spin cartridge.

3. Wash Procedure

Microfuge the spin cartridge assembly for 30 seconds to remove most of the non-bound protein. Add 0.5 ml of Wash Buffer **AC052-001-B** and spin again. Perform 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 **AC052-001-T2** supplied with the kit, as these have an extended hinge to accommodate the spin cartridge, and are required for the Elution step.

4. Elution

Transfer the cartridge to a clean collecting tube **AC052-001-T2**. Add 100 µl of Elution Buffer **AC052-001-E** and incubate for 2 minutes at room temperature with gentle agitation. Microfuge for 30 seconds. Remove the collecting tube (See Section 5) and add 25 µl Neutralization Buffer **AC052-001-N**.

Place the cartridge in a new collecting tube and add a further 100 µl of Elution Buffer **AC052-001-B** to the Protein A Resin. Incubate for 2 minutes at room temperature with gentle agitation. Spin and collect and neutralize as before.

Repeat the Elution procedure until all four clean collecting tubes have been used. The protein normally elutes in tubes 1 and 2 but you should confirm this using a test for protein (See Section 5) before pooling any of the tubes.

Pool the tubes with the most protein (this is 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.

5. Test for Protein

Wherever possible protein values should be determined using an absorbance at 280nm.

When other methods are used such as BCA or Bradford protein assays, determinations should be performed before the addition of the Neutralization Buffer **AC052-001-N**.
The Neutralization Buffer **AC052-001-N** 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 **AC052-001-E** 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 2.3-fold under-estimate of the IgG concentration.

Storage:

Storage of Kit: The kit is shipped at ambient temperature. Store the kit at 4°C upon receipt.

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

Pictures:

Figure 1: Spin cartridge / collecting tube assembly.