PROTOCOL

AbPure™ Mouse Antibody Purification System

Applicable to: 261-0005 1 mini purification
261-0010 3 mini purifications

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INTRODUCTION

Commercially available antibodies often contain substances (e.g. BSA and other proteins, glycine, tris and azide) that interfere in conjugation reactions. The AbPure™ Mouse Antibody Purification System quickly removes these contaminants. It can also be used to purify mouse antibodies from crude samples such as ascites fluid. The purification system can be used with antibody volumes from 0.1 ml to 0.5 ml. 20 to 150 µg of antibody can be purified in each run.

The method involves capturing the mouse antibody on AbPure™ Mouse resin which has a high affinity for mouse IgG molecules. Once the mouse antibody has bound to the AbPure™ Mouse resin, unwanted substances can be removed by simply washing the resin. The purified antibody is then eluted and neutralized.

The AbPure™ Mouse Antibody Purification System is not suitable for use with antibodies from other species.

The AbPure™ Mouse Antibody Purification System has been designed to be fully compatible with the InnovaCoat® GOLD and the LATEX conjugation kits (available separately), which allow the purified antibody to be conjugated rapidly.

KIT CONTENTS

- 1 or 3 vials of AbPure™ Mouse resin
- 1 vial of 10x AbPure™ Binding Buffer
- 1 vial of AbPure™ Wash Buffer
- 1 vial of AbPure™ Elution Buffer
- 1 vial of AbPure™ Neutralization Buffer
- 1 or 3 spin cartridge / collecting tube assemblies
- 4 or 12 additional collecting tubes

Not supplied: protein assay reagent

SHIPPING CONDITIONS

The kit is shipped at ambient temperature. Store the kit at 4°C upon receipt.

STORAGE OF MOUSE ANTIBODY

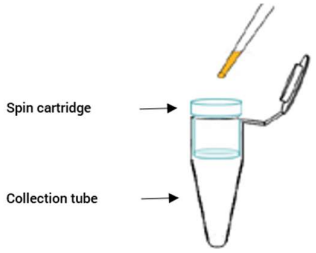
Store at 4°C. Other storage conditions (e.g. frozen at -70°C) may also be satisfactory. The sensitivity of any particular mouse antibody to freeze-thaw should be determined by experimentation on small aliquots.

INSTRUCTIONS

1. Preparation of AbPure™ Mouse resin

Add 0.3 ml of AbPure™ Wash Buffer to each vial of AbPure™ Mouse resin, mix by inversion for a few seconds and transfer to the spin cartridge (Figure 1). Spin for 30 seconds in a microfuge.

Figure 1 – Spin cartridge / collecting tube assembly



2. Incubate the sample with the resin

Add the appropriate amount of 10x AbPure™ Binding Buffer to the mouse antibody which corresponds to 1/10th of the sample volume. For example, if the sample volume is 200 µl, add 20 µl of 10X AbPure™ Binding Buffer. Pipette the sample into the spin cartridge and cap the tube. Pipette the sample into the spin cartridge and cap the tube. Incubate with mixing to maximize binding. This can be either at either 4ºC or room temperature overnight, or at room temperature for a minimum of 3 hours.

Note: The volume of mouse 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 (combined with the 10X AbPure™ Binding Buffer) with the AbPure™ Mouse resin in a larger vessel (e.g. 2 ml microcentrifuge tube) prior to transferring to the spin cartridge in several aliquots, spinning down excess liquid each time.

3. Wash procedure

Microfuge the spin cartridge assembly for 30 seconds to remove most of the non-bound protein. Add 0.5 ml of AbPure™ Wash Buffer and spin again. Perform the wash procedure a total of 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 microcentrifuge tubes (not supplied). Do not use the four (or twelve) collecting tubes supplied with the kit, as these have an extended hinge to accommodate the spin cartridge, and are required for the elution step.

4. Elute and neutralize the purified antibody

See Appendix before starting this step.
Transfer the cartridge to a clean collecting tube. Add 100 µl of AbPure™ Elution Buffer and incubate for 2 minutes at room temperature with gentle agitation. Microfuge for 30 seconds.

Remove the collecting tube (see Appendix) and add 11 µl AbPure™ Neutralization Buffer. The AbPure™ Neutralization Buffer should be added to the sample as soon as possible as long exposure to the low pH of the AbPure™ Elution Buffer can denature the antibody.

Place the cartridge in a new collecting tube and add a further 100 µl of AbPure™ Elution Buffer to the AbPure™ Mouse resin. Incubate for 2 minutes at room temperature with gentle agitation. Spin, 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 appendix) 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 mouse antibody you may choose to pool all tubes that contain protein, regardless of concentration.

COMPATIBILITY WITH LATEX CONJUGATION KITS

When using antibody purified with this kit in any of the LATEX Conjugation Kits, the eluted antibody must be at a concentration of at least 0.25 mg/ml for a successful conjugation. If the final antibody concentration is below this we recommend using the Antibody Concentration & Clean Up Kit for LATEX after this Mouse Antibody Purification System to concentrate the antibody and exchange the buffer to one more compatible with the LATEX Conjugation Kits.

COMPATIBILITY WITH INNOVACOAT GOLD KITS

For the InnovaCoat GOLD Conjugation Kits as long as the recovered antibody is sufficiently concentrated for the conjugation reaction the purified antibody is completely compatible, even if the antibody is eluted at concentrations below 0.25 mg/ml.

APPENDIX

Test for protein concentration

Suitable methods for protein concentration determination can be BCA or Bradford protein assay and absorbance measurement at 280nm.

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.

For the 280nm absorbance measurement, an extinction co-efficient of 1.4 is generally used for IgG – so a 1 mg/ml solution of IgG will give an absorbance value of 1.4 when measured with a 1 cm path length.

Note: if a low volume/amount of antibody has been added, the concentration of protein in the eluates will be low.

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TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: technical.enquiries@expedeon.com

For further information see our website: www.expedeon.com