PROTOCOL

AbSelect™ Antibody Purification System

Applicable to: 860-0005 1 purification
860-0010 3 Purifications

Release 1 © EXPEDEON.21/02/2018

INTRODUCTION

Commercially available antibodies often contain substances (e.g. BSA, glycine, tris, and azide) that interfere in labeling reactions. The AbSelect Purification System 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 0.1ml to 0.5ml. 20 to 500µg of antibody can be purified in each run.

The method involves capturing the antibody on the AbSelect Protein A resin. Protein A has a high affinity for the Fc regions of IgG molecules from a variety of species (see Appendix). Once the antibody has bound to the Protein A, unwanted substances can be removed by simply washing the resin. The antibody is then eluted and neutralized.

The AbSelect Antibody Purification System is fully compatible with both the Lightning-Link® and Lightning-Link® Rapid conjugation systems (available separately), which allow the purified antibody to be labeled with a hands-on time of under 30 seconds.

KIT CONTENTS

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

Not supplied: Protein assay reagent

SHIPPING AND STORAGE

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

STORAGE OF ANTIBODY

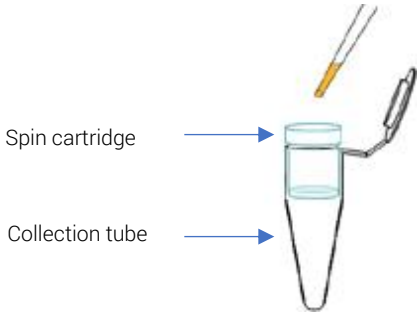
Store at 4°C. Other storage conditions (e.g. frozen at -70°C) may also be satisfactory. The sensitivity of any particular antibody to freeze-thaw should be determined by experimentation on small aliquots.

INSTRUCTIONS

1. Transfer the AbSelect Protein A resin to the spin cartridge

Add 0.3ml of Wash Buffer to the vial of Protein A 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 AbSelect 10x Binding Buffer to the antibody. The amount corresponds to 1/10th of the volume sample. For example, if the sample volume is 200µl, add 20µl of Binding Buffer. Pipette the sample into the spin cartridge and cap the tube. Incubate for 2 hours at room temperature with agitation or periodic shaking. Alternatively, incubate overnight at either 4°C or room temperature.

Note: The volume of antibody to be purified or cleaned up should be 0.1-0.5ml, though larger volumes may be processed by first incubating the antibody sample (combined with the Binding Buffer) with the Protein A resin in a larger vessel (e.g. 2ml eppendorf) prior to transferring to the spin cartridge in several aliquots, spinning down the excess liquid each time.

3. Wash the protein A resin

Microfuge the spin cartridge assembly for 30 seconds at 13,000g to remove most of the non-bound protein. Add 0.5ml of Wash Buffer and spin again. Repeat 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 eppendorfs (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 2 before starting this step.

The antibody is eluted in 100µl fractions. Transfer the cartridge to a clean collecting tube. Add 100µl of Elution Buffer and incubate for 2 minutes at room temperature with gentle agitation. Microfuge for 30 seconds at 13,000g. Remove the collecting tube (see section 3) and add 25µl Neutralization Buffer.

Place the cartridge in a new collecting tube and add a further 100µl of Elution Buffer 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 appendix 2) 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.

APPENDIX

Appendix 1. Protein A affinity for immunoglobulins

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Pig	IgG	High
Mouse	IgG_1	Low/Medium
Mouse	IgG_{2a}	High
Mouse	IgG_{2b}	High
Mouse	IgG_3	Low/Medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low

Appendix 2. Test for Protein

Wherever possible protein values should be determined using absorbance at 280nm. An extinction co-efficient of 1.4 is generally used for IgG – so a 1mg/ml solution of IgG will give an absorbance value of 1.4 when measured with a 1cm path length.

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

When other methods of determining IgG concentration are used such as BCA or Bradford protein assays, determinations should be performed before the addition of the Neutralization Buffer, as this can interfere with these reagents. Remove an aliquot for protein determination and neutralize the rest of the fraction immediately as the low pH of the elution buffer can denature the antibody.

RELATED PRODUCTS

Description	Prod. Code
AbSelect™ Antibody Concentration and Clean-Up Kit	861-0010

The Antibody Concentration and 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.

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