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AbSelect™ TCS Antibody Purification System

Applicable to:

862-0030 1 Purification (10 to 50ml tissue culture supernatant)

862-0500 3 Purifications (each 10 to 50ml tissue culture supernatant)

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Introduction

Protein A has a high affinity for the Fc regions of IgG molecules from a variety of species (see Appendix 1). AbSelect TCS is prepared by coupling purified Protein A to agarose beads. It can therefore be used to purify IgG fractions from hybridoma supernatants.

The method involves capture of the antibody on the AbSelect resin and the removal of unwanted substances using a simple wash procedure. The antibody is then eluted and neutralized. The AbSelect TCS 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 TCS Protein A resin

1 or 3 purification columns

1 bottle of 10x Binding Buffer

1 bottle of Wash Buffer

1 bottle of Elution Buffer

1 vial of Neutralization Buffer

1 or 3 concentrator spin columns and collection tubes

Shipping conditions

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

Amount of antibody that can be purified

The antibody to be purified should be in 10 to 50ml of tissue culture supernatant. Up to 5mg of antibody can be purified in each run.

Instructions

1. Prepare the tissue culture supernatant

Add the 10x Binding Buffer to the tissue culture supernatant. The volume to add is 1/10 of the volume of tissue culture supernatant. For example, for 50ml of tissue culture supernatant add 5ml of 10x Binding Buffer. Mix by inversion.

2. Incubate the sample with the resin

Add the AbSelect resin to the supernatant and incubate with mixing at room temperature for a minimum of 2 hours, or alternatively, leave overnight at either 4°C or room temperature, whilst still mixing. Use the supernatant to rinse the bottle to recover all the AbSelect resin.

3. Pack the column

Support the column in an upright position, and place a waste collection tube underneath (not provided). Carefully transfer the supernatant-resin mix into the column. Sample volumes of more than 10ml will have to be added in aliquots. The AbSelect TCS resin will collect in the bottom of the column.

The unwanted supernatant will pass through the column into the waste collection tube, and can be kept on ice until a successful outcome has been confirmed.

4. Wash procedure

Wash the column with Wash Buffer to remove any non-bound protein. Place another waste collection tube (not provided) under the column, and add 7ml wash buffer to the top of the column. Wait until it has all passed through, and then repeat the wash procedure a total of three times.

Keep the wash fractions again until a successful outcome has been confirmed.

Note: Wash the inner surface of the column to remove any residual starting material.

5. Elute and neutralize the purified antibody

See Appendix 2 before starting this step.

The antibody is eluted in 1ml fractions. Place a collecting tube under the column and add 1ml of Elution Buffer (see Appendix 2). Once all buffer has passed through the column, remove the collection tube and add 250µl of Neutralization Buffer. Cap the tube, mix and place to one side. Repeat the elution process three more times, each time neutralizing the sample as it is eluted. The neutralizing buffer must be added as soon as possible to avoid prolonged exposure to low pH which can result in denaturation of the IgG.

The protein normally elutes in tubes 1 and 2 but you should confirm this using a test for protein (Appendix 2) before pooling any of the tubes.

Antibody concentration (optional)

If the concentration of the recovered antibody is low then it can very quickly and easily be concentrated using the antibody concentrator.

1. Add antibody to the top of the spin cartridge.
2. Spin for 1 to 3 minutes* in a microfuge at a recommended maximum speed of 15,000g to reduce the buffer volume in the spin cartridge to between 50 and 100µl.
3. Repeat steps 1 and 2 as many times as is necessary to process the entire antibody to the desired concentration. It may be necessary to discard any excess buffer collected in the collection tube between spins.
4. Recover the concentrated antibody from the top of the spin cartridge.

NB. It is advisable not to spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and/or denaturation may occur.
*Spin times will vary depending on buffer composition and volume as well as centrifuge speed.

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.

Appendices

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.

For technical enquiries get in touch with our technical support team at:

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