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AbPure™ Magnetic Purification System

Applicable to:

265-0200 AbPure™ Magnetic Purification System

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Introduction

The AbPure™ Magnetic Purification System is a convenient method to purify antibodies at a small scale with high recoveries. It quickly removes substances often contained in commercially available antibodies (e.g. BSA and other proteins, glycine, tris, and azide) that interfere with conjugation reactions. It can be easily adapted to your needs allowing you to purify from 20 to 200µg of antibody and to reach the desired final antibody concentration. The antibody to be purified or cleaned up should ideally be in a volume of 20µl to 1ml.

The method involves capturing the antibody on the Protein A Magnetic Beads. Protein A has a high affinity for the Fc regions of IgG molecules from a variety of species (see Appendix 1). Once the antibody has bound to the Protein A, unwanted substances can be removed by simply washing the beads. The antibody is then eluted and neutralized.

The AbPure Magnetic Purification System has been designed to be compatible with our Lightning-Link®, Thunder-Link® PLUS, InnovaCoat® GOLD and LATEX conjugation kits (available separately), which allow the purified antibody to be conjugated rapidly.

Storage and shipping

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

Kit Contents

400ul of Protein A Magnetic Beads

1 vial of 10X Binding Buffer

1 bottle of Wash Buffer

1 vial of Elution Buffer

1 vial of Neutralization Buffer

Not supplied: magnetic stand, 1.5ml Eppendorf tubes, protein assay reagent, microfuge (optional)

Instructions

The procedure can be adjusted according to the amount of antibody to be purified. The buffers provided allow you to perform up to 10 purifications at the smallest scale. Follow Table 1 for instructions on volumes of beads and buffers.

Table 1. Volume reference table

Antibody amount	Beads volume	Elution volume	Neutralization volume
Up to 20µg	40µl	20µl	2µl
Up to 50µg	100µl	50µl	5µl
Up to 100µg	200µl	100µl	10µl
Up to 200µg	400µl	200µl	20µl

1. Wash Protein A Magnetic Beads

a. Mix the beads thoroughly by pipetting/vortexing before use. Add the appropriate amount of Protein A Magnetic beads to an Eppendorf tube according to Table 1.

Note: consider the antibody concentration required for your final application when planning the experiment (e.g. if you purify 10µg of antibody with 40µl beads, your final concentration will be ~0.5mg/ml). If you need 1mg/ml antibody it will be beneficial to add a slight excess of antibody to the beads.

b. Add 200µl of Wash Buffer and mix well by pipetting. Place the tube into a magnetic stand for 5-10 seconds to collect the beads and discard the supernatant. Repeat this washing step twice.

2. Incubate the sample with the magnetic beads

a. The minimum sample volume to add to the beads is 200µl. Add the appropriate amount of 10X Binding Buffer to the antibody which corresponds to one tenth of the sample volume. For example, if the sample volume is 200µl, add 20µl of 10X Binding Buffer. If the sample volume is lower than 200µl: dilute to 200µl with distilled water and add 20µl of 10X Binding Buffer.

b. Pipette the sample into the Eppendorf containing the washed beads and mix well by pipetting/vortexing. Incubate with mixing for 1 hour at room temperature to maximize binding.

3. Wash the Protein A Magnetic beads

a. Quickly spin the beads down in a microfuge at low rpm (<2000) for 10 seconds, in case they have accumulated on the top. Place the tube into a magnetic stand for 5-10 seconds to collect the beads and remove supernatant.

b. Add 200µl of Wash buffer and mix well by pipetting. Place the tube into the magnetic stand for 5-10 seconds to collect the beads and discard the supernatant. Repeat this washing step twice.

Note: the supernatant and wash fractions can be stored on ice until a positive outcome has been confirmed.

4. Elute and neutralize the purified antibody

a. Add the appropriate volume of Elution buffer to the beads according to Table 1.

b. Mix well by pipetting/vortexing and incubate with mixing for 5 minutes at room temperature.

c. Place the tube into the magnetic stand for 5-10 seconds

d. Collect the supernatant which contains the eluted antibody. The first fraction should contain most of the antibody.

e. Repeat steps a-d twice to collect two more separate elution fractions.

f. Neutralize your antibody with the appropriate volume of Neutralization Buffer, according to Table 1.

Note: The Neutralization Buffer should be added to the sample as soon as possible since long exposure to the low pH of the Elution Buffer can denature the antibody.

g. Measure the antibody concentration according to Appendix 2.

Storage of eluted 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.

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 1mg/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 Antibody Purification System to concentrate the antibody and exchange the buffer to one more compatible with the LATEX Conjugation Kits.

Compatibility with Lightning-Link® and InnovaCoat® GOLD Kits

If the recovered antibody is sufficiently concentrated for the conjugation reaction the purified antibody is completely compatible.

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 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, it is advisable to use a micro-volume UV/Vis spectrophotometer when working with a low antibody amount. The absorbance will proportionally change with the path length according to Beer-Lambert Law. 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 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.

Related products

For fast and effective magnetic beads separation, holds up to 8 Eppendorf tubes

265-1006 8-Tube Magnetic Stand

Technical Support

For technical enquiries get in touch with our technical support team at:
technical.enquiries@innovabiosciences.com
For further information see our website:
www.innovabiosciences.com