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ab270532 Magnetic Antibody Purification System

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 265-0200

View ab270532
Magnetic Antibody Purification System datasheet:
www.abcam.com/ab270532
(use www.abcam.cn/ab270532 for China, or www.abcam.co.jp/ab270532 for Japan)

For preparing antibodies for conjugation.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Magnetic Antibody Purification System (ab270532) is a convenient method to purify antibodies at a small scale with high recoveries. It quickly removes substances such as BSA, glycine, tris and azide that interfere with conjugation reactions. It can be easily adapted to your needs allowing you to purify from 20 to 200 µg 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 20 µL to 1 mL.

No dialysis of the antibody is needed prior to conjugation as interfering substances such as Tris, citrate and glycine have been replaced with conjugation-friendly alternatives.

The antibodies purified with the Magnetic Antibody Purification System are fully compatible with our Lightning-Link® Conjugation Kits, Oligonucleotide conjugation kit, Gold, Latex, Europium and Magnetic particle conjugation kits (available separately).

This sytem can directly replace any existing protein A purification kit and can be used to isolate IgG antibodies from crude mixtures.

- Less than 15 minutes hands on time.

- 70-90% antibody recovery.

- Up to 10 purifications at the smallest working scale.

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. **Do not freeze or store the resin at room temperature.** Freezing the suspension will damage the agarose beads.

Item	Quantity	Storage temperature
Protein A magnetic beads	400 µL	+4°C
10x Binding Buffer	1 bottle	+4°C
Wash Buffer	1 bottle	+4°C
Elution Buffer	1 bottle	+4°C
Neutralization Buffer	1 bottle	+4°C

Reagents are ready to use as supplied.

Materials Not Supplied: Magnetic Stand

3. Technical Considerations

3.1 Amounts of antibody to be purified:

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 the table below for instructions on volumes of beads and buffers.

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

3.2 Compatibility with Conjugation Kits

Antibodies purified using the Magnetic Antibody Purification System are fully compatible with our Lightning-Link®, Oligonucleotide, Gold, Latex, Europium and Magnetic particle conjugation kits, providing the antibody is purified and resuspended at a sufficient concentration for the conjugation reaction. We recommend a stock concentration of purified antibody of 1 mg/mL. For more information on required concentrations, consult the protocol for the applicable conjugation kit.

3.3 Protein A affinity for immunoglobulins:

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Pig	IgG	High
Mouse	IgG1	Low/Medium
Mouse	IgG2a	High
Mouse	IgG2b	High
Mouse	IgG3	Low/Medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low

3.4 Test for protein

Suitable methods for protein concentration determination can be BCA or Bradford protein assay and absorbance measurement at 280 nm. 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 280 nm 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 1 mg/mL solution of IgG will give an absorbance value of 1.4 when measured with a 1 cm path length.

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

4. Assay Procedure

4.1 Wash Protein A Magnetic Beads:

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

Add 200 µL 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.

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

4.2 Incubate the sample with the magnetic beads:

The minimum sample volume to add to the beads is 200 µL. 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 200 µL, add 20 µL

of 10X Binding Buffer. If the sample volume is lower than 200 µL then dilute to 200 µL with distilled water and add 20 µL of 10X Binding Buffer.

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.

4.3 Wash the Protein A Magnetic beads:

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.
Add 200 µL 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.

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

4.4 Elute and neutralize the purified antibody:

Add the appropriate volume of Elution buffer to the beads according to the table on page 4.

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

Place the tube into the magnetic stand for 5-10 seconds. Collect the supernatant which contains the eluted antibody. The first fraction should contain most of the antibody.

Repeat steps 6.4.1 – 6.4.4 twice to collect two more separate elution fractions.

Neutralize your antibody with the appropriate volume of Neutralization Buffer, according to the table on page 4.

Δ 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.*

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)