Version 6a

ab102784 Antibody Purification Kit (Protein A) Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 860-0005, 860-0010.

View ab102784
Antibody Purification Kit (Protein A) datasheet:
www.abcam.com/ab102784
(use www.abcam.cn/ab102784 for China, or www.abcam.co.jp/ab102784 for Japan)

For preparing antibodies for conjugation.

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

Table of Contents

1. Overview 2
2. Materials Supplied and Storage 3
3. Technical Considerations 4
4. Assay Procedure 6

1. Overview

Commercially available antibodies often contain substances (e.g. BSA, glycine, Tris, azide) that interfere in labeling reactions with enzymes or fluorophores. ab102784 quickly removes these contaminants. It can also be used to purify antibodies from crude samples such as ascites fluid or immune serum.

The method involves capture of the antibody on Protein A resin and the removal of unwanted substances by a simple wash procedure, which is carried out in a standard microfuge. The purified product is then eluted and neutralized.

***Δ Note**: This method cannot be used with samples containing relatively dilute antibody in large volumes (e.g. tissue culture supernatant). For larger volumes, we recommend the use of Antibody TCS Purification Kit (ab109207) or Antibody Serum Purification kit (ab109209).*

Antibodies purified using the Antibody Purification Kit (Protein A) are fully compatible with our Lightning-Link® Antibody Conjugation kits and our Oligonucleotide Conjugation Kit.

2. Materials Supplied and Storage

Store at +4°C upon receipt. **Do not freeze or store the resin at room temperature.**

Item	Quantity		Storage tempertur e
	1 x Test	3 x Test	
Spin Cartridge/Collecting Tube Assembly	1 unit	3 units	+4°C
Additional Collecting Tubes	4 units	12 units	+4°C
10x Binding Buffer	1 vial	1 vial	+4°C
Wash Buffer	1 vial	1 vial	+4°C
Elution Buffer	1 vial	1 vial	+4°C
Protein A resin	1 vial	3 vials	+4°C
Neutralization Buffer	1 vial	1 vial	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Recommended antibody quantities:

The antibody to be purified or cleaned up is ideally in a volume of 100 µL to 0.5 mL. 20 to 500 µg of antibody can be purified in each run.

3.2 Protein A affinity for immunoglobulins:

Protein A has a high affinity for the Fc regions of IgG molecules from a variety of species.

Species	Ig subclass	Binding to Protein A
Rabbit	IgG	High
Human	IgG_1	High
	IgG_2	High
	IgG_3	No affinity
	IgG_4	High
Pig	IgG	High
Mouse	IgG_1	Low/medium
	IgG_{2a}	High
	IgG_{2b}	High
	IgG_3	Low/medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low
	IgG_1	Low
	IgG_{2a}	Low
	IgG_{2b}	Low
	IgG_{2c}	Low

3.3 Antibody pre-conjugation considerations:

This kit can be used for preparing antibodies for conjugation. The antibody concentration for each Conjugation Kit has been optimised. Before starting the elution step of this purification procedure, please refer to the relevant Lightning-Link® Conjugation Kit datasheet or protocol for the recommended antibody concentration and find more general information about antibody conjugation at www.abcam.com/conjugationFAQs.

3.4 Test for protein concentration:

Wherever possible, protein values should be determined using an absorbance at 280 nm. 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.*

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.

4. Assay Procedure

4.1 Transfer of the Protein A resin to the spin cartridge:

Add 0.3 mL of Wash Buffer to the vial of Protein A resin, mix by inversion for a few seconds and transfer to the spin cartridge. Spin for 30 seconds in a microfuge.

4.2 Incubation of Sample with Resin:

To the antibody, add an appropriate amount of 10x Binding Buffer. The amount corresponds to 1/10th of the sample volume. For example, if the sample volume is 200 µL, add 20 µL of 10x 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.

*Δ **Note:** The volume of antibody to be purified or cleaned up should ideally be 0.1-0.5 mL, 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. 2 mL tube) prior to transferring to the spin cartridge in several aliquots, spinning down the excess liquid each time.*

4.3 Wash Procedure:

Microfuge the spin cartridge assembly for 30 seconds at 13,000 x *g* to remove most of the non-bound protein. Add 0.5 mL of Wash Buffer and spin again. Repeat the wash procedure three times.

*Δ **Note:** Save the non-bound and wash fractions by transferring the material from the collecting tube after each spin to a set of new tubes (not supplied). Do not use the four collecting tubes supplied with the kit, as these have an extended hinge to accommodate the spin cartridge and are required for the elution step.*

4.4 Elution:

Please see the Technical Considerations sections 3.3 and 3.4 before starting this step.

Transfer the cartridge to a clean collecting tube. Add 100 µL of Elution Buffer and incubate for 2 minutes at room temperature with gentle agitation. Microfuge for 30 seconds 13,000 x *g*. Remove the collecting tube and add 25 µL Neutralization Buffer to the tube.

Place the cartridge in a new collecting tube and add a further 100 µL 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 concentration (see Technical Consideration section 3.4) before pooling any of the tubes.

Pool the tubes with most protein (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.

4.5 Antibody storage:

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.

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)