Version 7e

ab109209 Serum Antibody Purification Kit (Protein A) Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 863-0030, 863-0500.

View ab109209
Serum Antibody Purification Kit (Protein A) datasheet:
www.abcam.com/ab109209
(use www.abcam.cn/ab109209 for China, or www.abcam.co.jp/ab109209 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

The Serum Antibody Purification kit (Protein A) (ab109209) is prepared by coupling highly purified Protein A to agarose beads and can therefore be used to purify IgG fractions from both serum and ascites fluid.

The antibody is captured on the resin and unwanted substances are removed by a simple wash procedure. The purified product is then eluted and neutralized.

Antibodies purified using the Serum 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 temperature
	1 x Test	3 x Test	
Purification Column	1 unit	3 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
Serum 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:

Each column can purify up to 20 mg of antibody. The volume of the sample required will depend on the host species.

Total IgG levels in normal serum and ascites fluid:

Species	Normal Range IgG (mg/mL)	Suitable Volume for Product (mL)
Rabbit	12-15	1.3-1.7
Human	7-23	0.9-2.9
Mouse	2-5	4-10
Sheep/Goat	18-24	0.8-1.1
Rat	5-7	2.9-4
Ascites Fluid	0.5-5	4-40

3.2 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.

3.3 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

3.4 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.

When using Bradford-type reagents it is important to use an IgG standard curve. Failure to do this will result in incorrect antibody levels being calculated. If IgG is not available then a BSA standard curve can be used, but the IgG levels will be under-estimated by a factor of 2.3.

4. Assay Procedure

4.1 Serum or Ascites Fluid Preparation:

Add the 10x Binding buffer to the serum or ascites fluid. The volume to add is 1/10 of the volume of the sample. For example, for 5 mL of serum add 0.5 mL of 10x Binding Buffer and mix by inversion.

*Δ **Note:** For sample with volumes of fewer than 5 mL, dilute the sample with Wash Buffer to 5 mL before adding the 10x Binding Buffer.*

4.2 Incubation of Sample with Resin:

Add the Protein A resin to the prepared supernatant and incubate with continuous mixing at room temperature for a minimum of 2 hours. Alternatively, incubate overnight at either +4°C or room temperature. Use the supernatant to rinse the glass vial to recover all Protein A resin.

4.3 Transfer of the Resin into the Column:

Place a collection tube (not included) under the column.
Carefully pour the serum-Resin mix into the column. Sample volumes of more than 10 mL have to be added in aliquots. The resin will stack at the bottom of the column. Unwanted supernatant will pass through the column and can be kept on ice until a successful outcome has been confirmed.

4.4 Wash Procedure:

Gently add 7 mL of Wash Buffer to the top of the resin and allow this to pass through the column. Perform this step a total of three times. This will remove any unbound proteins, leaving only IgG bound to the resin.

4.5 Elution:

See Technical Considerations sections 3.2 and 3.4 before starting this step.

*Δ **Note:** Elute the antibody in 1 mL fractions.*

Place a collecting tube (not included) under the column and add 1 mL of Elution Buffer. Collect 1 mL of liquid in the tube and add 0.25 mL of Neutralization Buffer. Cap the tube and place to one side.

Repeat the elution process three more times, each time neutralizing the sample as it is eluted.

Δ Note: *The Neutralization Buffer must be added to the sample as soon as possible to avoid prolonged exposure to low pH which can result in the denaturation of the IgG.*

Δ Note: *The IgG normally elutes in Tubes 1 and 2 but you should confirm this using a test for protein before pooling any of the tubes (see the Technical Considerations section 3.4). If more than 2 tubes are strongly positive, it is possible that you have used too much sample for the purification.*

4.6 Antibody Concentration (optional):

If the concentration of the recovered antibody is low then it can quickly and easily be concentrated using a clean spin cartridge.

Add the antibody to the top of the spin cartridge.

Spin for 1-3 minutes in a microfuge at maximum speed of 15,000 x *g* to reduce the buffer volume in the spin cartridge to 50-100 µL (Spin times will vary depending on the buffer composition and volume as well as centrifuge speed).

Repeat these 2 steps 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 tubes between spins.

Recover the concentrated antibody from the top of the spin cartridge.

Δ ***Note:*** *It is advisable not to spin the antibody dry as reconstitution will be difficult and there will be significant antibody loss and/or denaturation.*

4.7 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:
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