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ab128751 Serum Antibody Purification Kit (Protein G) Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 893-0030, 893-0500

View ab128751 Serum Antibody Purification Kit (Protein G) datasheet:

www.abcam.com/ab128751

(use www.abcam.cn/ab128751 for China, or www.abcam.co.jp/ab128751 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

Protein G has a high affinity for the Fc regions of IgG molecules from a variety of species. ab128751 Protein G Serum resin is prepared by coupling purified Protein G to agarose beads. It can therefore be used to purify IgG fractions from both serum and ascites fluid. The antibody is captured on the Protein G resin and unwanted substances are removed by a simple wash procedure. The purified product is then eluted and neutralized.

The antibodies purified with Protein G Serum Antibody Purification Kit are fully compatible with our Lightning-Link® antibody conjugation kits and Oligonucleotide Conjugation Kit.

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
	1 TEST	3 TESTS	
Serum Protein G resin	1 bottle	3 bottles	+4°C
Purification Columns	1 column	3 columns	+4°C
10x Binding Buffer	1 bottle	1 bottle	+4°C
Wash Buffer	1 bottle	1 bottle	+4°C
Elution Buffer	1 bottle	1 bottle	+4°C
Neutralization Buffer	1 bottle	1 bottle	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Amount of antibody that can be purified:

Up to 10 mg of antibody can be purified in each run. The volume of the sample required will depend on the host species.

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 IgG levels in normal serum and ascites fluid:

Species	Normal Range IgG (mg/ml)	Suitable Volume for Product (ml)
Rabbit	12-15	0.7-0.8
Human	7-23	0.4-1.4
Mouse	2-5	2-5
Sheep	18-24	0.4-0.6
Goat	18-24	0.4-0.6
Rat	5-7	1.4-2
Ascites Fluid	0.5-5	2-20

3.4 Protein G affinity for immunoglobulins:

Species	Ig	Binding strength
Rabit	IgG	High
Human	IgG (normal)	Very high
Human	IgG_1	Very high
Human	IgG_2	Very high
Human	IgG_3	Very high
Human	IgG_4	Very high
Human	IgM	None
Human	IgA	None
Human	IgE	None
Mouse	IgG_1	Very high
Mouse	IgG_{2a}	Very high
Mouse	IgG_{2b}	Medium
Mouse	IgG_3	Medium
Goat	IgG	Medium
Sheep	IgG	Medium
Rat	IgG_1	Low
Rat	IgG_{2a}	Very high
Rat	IgG_{2b}	Medium
Rat	IgG_{2c}	Medium

3.5 Test for protein:

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 1cm path length.

When other methods 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 tissue culture supernatant (add 1/10 of the volume of tissue culture supernatant). For example, for 50 mL of tissue culture supernatant add 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 Incubating sample with the resin:

Add the protein G resin to the prepared supernatant and incubate with mixing at RT 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 G resin.

4.3 Packing 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. Repeat this step a total

of three times. This will remove any unbound proteins, leaving only IgG bound to the resin.

4.5 Elution:

Please see Technical Consideration section on Test for protein before starting this step.

Elute the antibody in 1 mL fractions. Place a set of collection tubes under the column ready for elution. Add 1 mL of Elution Buffer to the column and collect the liquid

Remove the collection tube from underneath the column and add 250 µL 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.

Δ Note: *The eluted antibody must be neutralized as soon as possible to avoid prolonged exposure to low pH of Elution Buffer which can result in denaturation of the IgG.*

Δ Note: *The protein normally elutes in tubes 1 and 2, by spin-purification, and in tubes 2 and 3 by gravity-purification, but you should confirm this using a test for protein before pooling any of the tubes.*

4.6 Antibody storage:

Store at 4°C. Other storage conditions (e.g. frozen at -70°C) may also be satisfactory. The sensitivity of any antibody to freeze thaw should be determined by experimentation on small aliquots.

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