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ab128747 Antibody Purification Kit (Protein G) Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 890-0010, 890-0005

View ab128747
Antibody Purification Kit (Protein G) datasheet:
www.abcam.com/ab128747
(use www.abcam.cn/ab128747 for China, or www.abcam.co.jp/ab128747 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

Commercially available antibodies often contain substances (e.g. BSA, glycine, tris, azide) that interfere in labeling reactions with enzymes or fluorophores. ab128747 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 capturing the antibody on the Protein G resin. Protein G has a high affinity for the Fc regions of IgG molecules from a variety of species. Once the antibody has bound to the Protein G, unwanted substances can be removed by simply washing the resin.

Antibodies purified with the Antibody Purification Kit (Protein G) 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	
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
Additional Collecting Tubes	4 units	12 units	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Amount of antibody that can be purified:

The antibody to be purified or cleaned up should be in a volume of 100 to 500 µL. 20 to 300 µg of antibody can be purified in each run.

3.2 Antibody pre-conjugation considerations:

This kit can be used for preparing antibodies for conjugation. 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 G affinity for immunoglobulins:

Species	Ig	Binding Strength
Rabbit	IgG	High
Human	IgG	High
Mouse	IgG_1	Medium
Mouse	IgG_{2a}	High
Mouse	IgG_{2b}	Medium
Mouse	IgG_3	Medium
Mouse	IgM	No binding
Goat	IgG	High
Sheep	IgG	High
Rat	IgG_1	Weak/Medium
Rat	IgG_{2a}	High
Rat	IgG_{2b}	Weak/Medium
Rat	IgG_{2c}	Weak/Medium

3.4 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 Reconstitution of Protein G

Add 300 µL of Wash Buffer to each vial of Resin, mix by inversion for a few seconds and transfer to the spin cartridge. Spin for 30 seconds in a microfuge.

4.2 Incubating sample with the resin:

To the antibody, add an appropriate amount of 10x Binding Buffer. For example, if the sample volume is 200 µL, add 20 µL of Binding Buffer. Pipette the sample into the spin cartridge and cap the tube. Incubate for a minimum of 2 hours 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 100-500 µL, though larger volumes may be processed by first incubating the antibody sample with the protein G resin in a larger vessel (e.g. 2 mL tube) prior to transferring to the spin cartridge.*

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 500 µL 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 eppendorfs (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 Consideration section on Test for protein before starting this step.

Transfer the cartridge to a clean collecting tube. Add 100 µL of Elution Buffer and incubate for 2 mins at room temperature with gentle agitation. Microfuge for 30 seconds at 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 G resin. Incubate for 2 mins at room temperature with gentle agitation. Spin and collect and neutralize as before.

Repeat the elution procedure until all four clean collecting cups have been used.

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.

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 but you should confirm this using a test for protein before pooling any of the tubes.

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 antibody to freeze thaw should be determined by experimentation on small aliquots.

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