ab109209 – Antibody Serum Purification Kit (Protein A) Protocol

For preparing antibodies for conjugation

The components of **ab109209** are fully compatible with our Conjugation kits however they are not compatible with our GOLD Antibody conjugation kits. To purify antibodies for use with our GOLD conjugation kits, please use our Gold antibody purification kit (ab204909).

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

Version 6 Last Updated 4 April 2019

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1. Introduction

The Antibody Serum 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.

The components of ab109209 are fully compatible with our antibody conjugation kits.

ab109209 is not suitable for goat antibody purification.

2. Kit Contents

	1 x Test	3 x Test
Purification Column	1 unit	3 units
10x Binding Buffer	1 vial	1 vial
Wash Buffer	1 vial	1 vial
Elution Buffer	1 vial	1 vial
Protein A resin	1 vial	3 vials
Neutralizer	1 vial	1 vial

3. Reagents Required, Not supplied

- Antibody to be purified

4. Storage and Handling

Kits are shipped at ambient temperature in a tamper-evident polypropylene container. Store at 4°C upon receipt.

5. Recommended antibody quantities

Each column can purify up to 20mg of antibody.

The volume of the sample required will depend on the host species

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	2.8-4
Ascites Fluid	0.5-5	4-40

6. Purification Protocol

1. **Serum or Ascites Fluid Preparation**

   Add the 10x Binding buffer to the serum or ascites fluid (add 1/10 of the volume of sample). For example, for *5ml* of serum add 0.*5ml* of 10x Binding Buffer and mix by inversion.

Note: *For sample with volumes of fewer than 5ml, dilute the sample with wash buffer to 5ml before adding the 10x Binding Buffer*

2. **Incubation of Sample with Resin**

Add the protein A 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 A resin.

Note: *Protein A resin has less affinity for sheep antibodies than for mouse/rabbit antibodies, and this will affect the binding capacity.*

3. Packing of the Column

Carefully pour the serum-resin mix into the column. Sample volumes of more than 10ml 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. Wash Procedure

Wash the column with 7ml of Washing buffer to remove any non-bound protein. Repeat the washing step three times.

5. Elution

Note: *Elute the antibody in 1ml fractions*

Place a set of collection tubes under the column ready for elution. Add 1ml of Elution Buffer to the column and collect the liquid

Remove the collection tube from underneath the column and add 250µl of Neutralizing 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.

Note: *The Neutralizing 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.*

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.

6. Antibody Concentration (optional)

This step should be performed using an additional kit ab102778 (Antibody Concentration Kit).

Add the antibody to the top of the spin cartridge.

Spin for 1-3 minutes in a microfuge at maximum speed of 15000g 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 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.

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.*

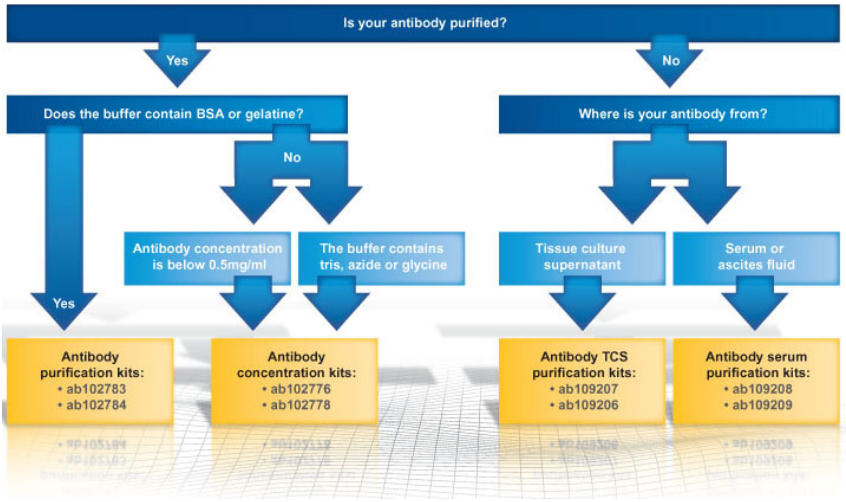
7. Antibody pre-conjugation considerations

The purified antibody to be labeled should ideally be in 10-50mM amine-free buffer (e.g. MES, MOPS, HEPES, PBS), pH range 6.5 to 8.5. If the buffer is more concentrated or outside this pH range contact the technical support team.

Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars have no effect on conjugation efficiency. Azide (0.02 to 0.1%) and BSA (0.1 to 0.5%) have little or no effect. Glycerol up to 50% has no effect.

Avoid buffer components that are nucleophilic, as these may react with chemicals. Primary amines (e.g. amino acids or ethanolamine) and thiols (e.g. mercaptoethanol or DTT) fall within this class (Note: Tris-based buffers should be avoided). If your buffer contains primary amines and/or thiols, you should consider using our Concentration and Purification Kits.

Antibody concentration and purification kit selection guide:


Is your antibody purified?
Yes
No
Does the buffer contain BSA or gelatine?
No
Where is your antibody from?
Antibody concentration is below 0.5mg/ml
The buffer contains tris, azide or glycine
Tissue culture supernatant
Serum or ascites fluid
Yes
Antibody purification kits:
• ab102783
• ab102784
Antibody concentration kits:
• ab102776
• ab102778
Antibody TCS purification kits:
• ab109207
• ab109206
Antibody serum purification kits:
• ab109208
• ab109209

8. Storage of Antibody

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.

9. Test for Protein

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

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

10. General FAQs

1. What is the optimal starting concentration for the antibody?

The antibody conjugation kit allows antibody labeling to be performed on a microgram to milligram scale. The amount of antibody should correspond to molar ratios between 1:4 and 1:1 of antibody to conjugate. Based on their molecular weights (e.g. 160kDa for the antibody versus 40kDa for the conjugate), 100-400µg of conjugate can be added to 100µg of antibody. Antibody concentrations of 0.5-5mg/ml give optimal results. We recommend using 10µl, 100µl and 1ml of antibody solution with the 10µg, 100µg and 1mg kit formats, respectively. The antibody concentration for each conjugation kit has been optimized. Please refer to the relevant datasheet or protocol for the recommended antibody concentration.

2. Do I need to purify the antibody before using the conjugation kit?

Yes. The antibody labeling chemistry involves free amine groups. Most proteins/peptides have lysine and/or alpha-amino groups, therefore, any protein/peptide present in the solution will also be labeled. We recommend purifying your antibodies before performing the conjugation. Ascites fluid, serum or hybridoma

culture media should be avoided. View compatible and incompatible buffers in question 5**.**

3. **Are conjugation kits suitable for proteins and secondary antibodies?**

   Yes. The labeling chemistry involves free amines present in lysines and at the N-terminus of a protein. All antibodies have multiple free amine groups and most proteins have lysine and/or alpha-amino groups. As long as lysines are present, secondary antibodies and proteins will be labeled with the conjugation kits. However, they have not yet been specifically tested with secondary antibodies and proteins.

4. **What buffers can be used?**

   We recommend using Hepes, MES, MOPS and phosphate-based buffers or any other amine-free buffer. Conjugation reactions can also be prepared in the presence of up to 20mM Tris buffer with almost no reduction in coupling efficiency. Once the reaction is complete, the conjugated antibody can be diluted in any buffer compatible with both label and antibody.

5. **Which buffer additives can be used and what should be avoided?**

   Additives such as salts (e.g. NaCl), sugars (e.g. sucrose) and chelators (e.g. EDTA) have no effect on the labeling reaction. We

recommend avoiding nucleophiles such as amino acids (e.g. glycine), blockers (e.g. ethanolamine) and thiols (DTT, mercaptoethanol) that might deactivate the chemical which covalently links the conjugate to the antibody.

Compatible additives:

- up to 20mM Tris
- up to 0.5% BSA
- up to 0.1% gelatin
- up to 0.1% sodium azide
- PBS pH7.4
- up to 50% glycerol
- 0.15M sodium chloride
- 50mM HEPES
- 0.02M potassium phosphate
- 0.001% Tween
- Proclin 300
- 5% Trehalose

Incompatible additives:

- 60mM citrate + 150mM Tris pH7.8
- Urea
- 50mM Imidazole
- Glycine
- Ethanolamine
- DTT
- Mercaptoethanol

6. How do I remove additives from the antibody storage buffer?

Our Antibody Concentration and Purification kits remove additives with ease and provide a ready-to-use antibody solution compatible with the conjugation kit.

The Antibody Concentration kit allows an easy concentration and reduction of azide, glycine and Tris.

The Antibody Purification Kit quickly removes BSA, glycine, Tris, azide etc. and can also be used to purify antibodies from ascites fluid or immune serum.

For further FAQs visit: www.abcam.com/conjugationFAQS

11. Publications using Conjugation kits

Label: Cy3® Application: FRET
Effects of pH on molecular mechanisms of chitosane-integrin interactions and resulting tight-junction disruptions
Hsu LW*, et al, Biomaterials, Jan 2013, 34(3):784-793, PMID: 23103155*

Label: R-PE Application: Immunocytochemistry and *In-vivo* imaging*
Intraoperative Imaging of Metastatic Lymph Nodes Using a Fluorophore-conjugated Antibody in a HER2/neu-expressing Orthotopic Breast Cancer Mouse Model
Wu J *et al, Anticancer Res, Feb 2013, 33(2):419-24, PMID: 23393332*

*N.B. No product warranty for this application

Label: APC/Cy7® Application: Flow cytometry
APR-246/PRIMA-1^{MET} rescues epidermal differentiation in skin keratinocytes derived from EEC syndrome patients with p63 mutations
Shen J, *et al, Proc Natl Acad USA, Feb 2013, 110(6):2157-62, PMID: 23355676*

Label: FITC Application: Flow cytometry and cellular assay
Constitutive dimerization of glycoprotein VI (GPVI) in resting platelets is essential for binding to collagen and activation in flowing blood.
Jung SM, *et al, J Biol Chem, Aug 2012, 287(35):30000-13, PMID: 22773837*

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China and Asia Pacific
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