

ab204910 Mouse Antibody Purification Kit - Nanoparticles

A product of Expedeon, an
Abcam company

Applicable to Expedeon product codes 261-0005, 261-0010.

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Nanoparticles datasheet:

www.abcam.com/ab204910

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For purification of antibodies.

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

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1. Overview

Mouse Antibody Purification Kit - Nanoparticles (ab204910) removes contaminants often found in commercial antibodies (e.g. BSA, glycine, tris and azide) that interfere in conjugation reactions with enzymes or fluorophores. It can also be used to purify mouse antibodies from crude samples such as ascites fluid.

The method involves capturing the mouse antibody on Mouse Resin which has a high affinity for mouse IgG molecules, and removing unwanted substances by a simple wash procedure, which is performed in a standard microfuge. The purified antibody is then eluted and neutralized.

Antibodies purified using the Mouse Antibody Purification Kit – Nanoparticles are fully compatible with our Gold antibody conjugation kits, Magnetic particle conjugation kits, Latex and Europium conjugation kits (available separately).

Δ Note: *Mouse Antibody Purification Kit - Nanoparticles kit is not suitable for use with antibodies from other species. It should be noted that the binding strength of bovine IgG to the Mouse Resin is negligible.*

2. Materials Supplied and Storage

Kits are shipped at ambient temperature in a tamper-evident polypropylene container. Store kit 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 | |
| Mouse Resin | 1 Vial | 3 Vials | +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 |
| Neutralization Buffer | 1 Vial | 1 Vial | +4°C |
| Spin Cartridge/collecting tube assembly | 1 | 3 | +4°C |
| Additional Collecting tubes | 4 | 12 | +4°C |

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Amount of mouse antibody that can be purified:

The antibody to be purified or cleaned up is ideally in a volume of 100 μL to 500 μL . 20 to 150 μg of antibody can be purified in each run.

3.2 Compatibility with Nanoparticle Conjugation Kits:

Mouse antibodies purified using the Mouse Antibody Purification Kit – Nanoparticles are fully compatible with our Gold, Latex, Europium and Magnetic particle conjugation kits, providing the antibody is purified and resuspended at a sufficient concentration for the conjugation reaction. We recommend a stock concentration of purified antibody of 1 mg/mL. For more information on required concentrations, consult the protocol for the applicable conjugation kit.

3.3 Test for protein concentration:

Suitable methods for protein concentration determination can be BCA or Bradford protein assay and absorbance measurement at 280nm.

When using Bradford-type reagents it is important to use an IgG standard curve. The absorbance generated by this type of reagent is dependent on the protein used. For example using a BSA standard curve to determine the protein concentration of an IgG solution will result in a 2.3-fold underestimate of the IgG concentration.

For the 280nm absorbance measurement, an extinction coefficient 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. Note: if a low volume/amount of antibody has been added, the concentration of protein in the eluates will be low.

4. Assay Procedure

4.1 Reconstitution of Mouse Resin:

Add 300 μL of Wash Buffer to each vial of Mouse 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 mouse antibody, add an appropriate amount of 10X Binding Buffer which corresponds to 1/10th of the sample volume. 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 with mixing to maximize binding. This can be either at either 4°C or room temperature overnight, or at room temperature for a minimum of 3 hours.

***Δ Note:** The volume of mouse 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 (combined with the 10X AbPure™ Binding Buffer) with the Mouse Resin in a larger vessel (e.g. 2 mL tube) prior to transferring to the Spin Cartridge in several aliquots, spinning down excess liquid each time.*

4.3 Wash procedure:

Microfuge the Spin Cartridge assembly for 30 seconds 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 microfuge tubes (not supplied). Do not use the four/twelve 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 and neutralization of the purified antibody:

Please see Technical Considerations sections 3.2 and 3.3 before starting this step.

Transfer the cartridge to a clean collecting tube. Add 100 μ L of elution buffer and incubate for 2 minutes at room temperature with gentle agitation. Microfuge for 30 seconds. Remove the collecting tube and add 11 μ L Neutralization Buffer to the tube. The Neutralization Buffer should be added to the sample as soon as possible as long exposure to the low pH of the Elution Buffer can denature the antibody.

Place the cartridge in a new collecting tube and add a further 100 μ L of Elution buffer to the Mouse Resin. Incubate for 2 minutes at room temperature with gentle agitation. Spin, 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 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 Storage of mouse antibody:

Store at 4°C. Other storage conditions (e.g. frozen at -80°C) may also be satisfactory. The sensitivity of any particular antibody to freeze-thaw should be determined by experimentation on small aliquots.

Technical Support

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