abcam

Product datasheet

Antibody Serum Purification Kit (Protein A) ab109209

1 References

Overview

Product name Antibody Serum Purification Kit (Protein A)

Product overview **Antibody Serum Purification kit (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.

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).

Notes

ab109209 is not suitable for goat antibody purification.

ab109209 contains 1-3 columns, and each can purify up to **20mg** of antibody per run.

The volume of sample required will depend on the host species (See Table below):

Species	Normal range IgG(mg/ml)	Suitable Volumes 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

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 samples volumes of less than 5ml, dilute the sample with wash buffer to 5ml before adding the 10x Binding Buffer.

2. Incubate the sample with resin

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

Please note that protein A resin has less affinitiy for sheep antibodies than for mouse/rabbit antibodies, and this will affect the binding capacity.

3. Packing of the column

Carefully pour the sample-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.

NOTE: wash the inner surface of the column to remove any residual starting material.

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 through-out 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.

The Neutralizing buffer must be added as soon as possible to the sample to avoid prolonged expossure to low pH which can result in 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.

Antibody Concentration (optional)

If the concentration of the recovered antibody is low then it can very quickly and easily be concentrated using our Concentration kit (ab102778).

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.

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

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	3 tests	1 tests
10x Binding Buffer	1 unit	1 unit
Elution Buffer	1 unit	1 unit
Neutralizer	1 unit	1 unit
Protein A resin	3 units	1 unit
Purification Column	3 units	1 unit
Wash Buffer	1 unit	1 unit

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