ab270562
Rat TCS Antibody Purification Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 842-0500.

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Rat TCS Antibody Purification Kit datasheet:
www.abcam.com/ab270562
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For the purification of rat IgG fractions from tissue culture supernatants..

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

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1. Overview

Rat TCS Antibody Purification Kit (ab270562) is designed for the purification of rat IgG fractions from tissue culture supernatants. Rat Resin has a very high affinity and specificity for rat IgG molecules. The Rat TCS Antibody Purification Kit can be used to purify rat IgG fractions from hybridoma supernatants. The binding strength of bovine IgG to the Rat Resin is negligible; therefore, it can be used to selectively recover rat IgG from TCS samples.

The method involves capture of the rat antibody on the Rat resin and removal of unwanted substances using a simple wash procedure. The purified product is eluted and neutralized. Rat TCS Antibody Purification Kit is not suitable for use with antibodies from other species.

Antibodies purified using the Rat TCS Antibody Purification Kit are fully compatible with our Lightning-Link® Antibody Conjugation kits and our 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
Rat Resin	3 vials	+4°C
Spin cartridge / collecting tube assemblies	3 units	+4°C
10x Binding Buffer	1 vial	+4°C
Wash Buffer	1 vial	+4°C
Elution Buffer	1vial	+4°C
Neutralization Buffer	1 vial	+4°C
Additional collection tubes	12 tubes	+4°C
Concentrator spin columns	3 units	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Recommended antibody quantities:

Prepare tissue culture supernatant using standard techniques. The rat antibody to be purified should be in 10 to 50 mL of tissue culture supernatant. Up to 600 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. 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 Test for protein concentration:

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 1 cm path length.

Δ Note: *If a low volume/amount of antibody has been added, the concentration of protein in the eluates will be low.*

When other methods of determining IgG concentration 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.

4. Assay Procedure

4.1 Rat tissue culture supernatant preparation for binding:

Add the 10x Binding Buffer to the rat tissue culture supernatant. The volume required is 1/10 of the volume of tissue culture supernatant. For example, to 10 mL of tissue culture supernatant add 1 mL of 10x Binding Buffer and mix by inversion.

4.2 Incubation of Sample with Resin:

Add the Rat Resin to the supernatant and incubate with mixing at room temperature for a minimum of 2 hours. Alternatively, incubate overnight at either +4°C or room temperature. Use the supernatant to rinse the vial to recover all of the Rat Resin

4.3 Transfer of the Rat Resin into the spin cartridge:

Spin the solution for 3 minutes in a centrifuge at 3,000 x *g* to spin down the resin. Remove the supernatant as this will contain only cell debris and unwanted cell proteins. It is best not to discard the supernatant until a successful purification has been obtained.

Resuspend the resin in 500 µL of Wash Buffer and transfer the suspension to the spin cartridge. Spin for 30 seconds at 15,000 x *g*.

4.4 Wash Procedure:

Add 0.5 mL of Wash Buffer to the tube used in 7.3 to recover any residual resin and then transfer the solution to the spin cartridge. Spin for 30 seconds at 15000 x g.

Add a further 0.5 mL of Wash Buffer and spin for 30 seconds at 15,000 x g, then repeat this once more.

Δ Note: *Save the wash fractions by transferring the material after each spin from the collecting tube to a set of tubes. Keep them until a successful outcome has been confirmed.*

Δ ***Note:*** *Do not use the 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.*

4.5 Elution:

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

Transfer the spin cartridge to a clean collecting tube. Add 200 µL of Elution Buffer and incubate for 10 minutes at room temperature with gentle agitation. Microfuge for 30 seconds at 15,000 x *g*. Remove the collection tube and add 50 µl of Neutralization Buffer and mix. Repeat the elution process with a fresh collection tube three more times, each time neutralizing the sample as it is eluted.

Δ ***Note:*** *The protein normally elutes in tubes 1 and 2 but this should be confirmed by using a test for protein concentration (see Technical Considerations section 3.3) before pooling the tubes.*

4.6 Antibody Concentration (optional):

If the concentration of the recovered rat antibody is low then it can quickly and easily be concentrated using a clean spin cartridge.

Add the rat antibody to the top of the spin cartridge.

Spin for 1-3 minutes in a microfuge at maximum speed of 15,000 x *g* 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 entire rat antibody to the desired concentration. It may be necessary to discard any excess buffer collected in the tubes between spins.

Recover the concentrated rat antibody from the top of the spin cartridge.

Δ ***Note:*** *It is advisable not to spin the rat antibody dry as reconstitution will be difficult and there will be significant antibody loss and/or denaturation.*

4.7 Antibody storage:

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

Technical Support

For all technical or commercial enquiries please go to:

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