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ab270554 TCS Antibody Purification Kit - Nanoparticles

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 264-0030, 264-0500

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TCS Antibody Purification Kit - Nanoparticles datasheet:
www.abcam.com/ab270554
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For the purification of 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

TCS Antibody Purification Kit - Nanoparticles (ab270554) is designed for the purification of IgG fractions from tissue culture supernatants. Protein A resin is prepared by coupling purified Protein A to agarose beads.

The method involves capture of the antibody on the resin and the removal of unwanted substances using a simple wash procedure. The antibody is then eluted and neutralized. The kit can be used in its rapid version by spin-purification in a centrifuge, or by gravity-flow purification. The antibody to be purified should be in 10 mL to 50 mL of tissue culture supernatant (TCS). Up to 5 mg of antibody can be purified in each run.

The antibodies purified using TCS Antibody Purification Kit – Nanoparticles are fully compatible with our Gold, Latex, Europium and Magnetic particle conjugation kits (available separately).

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 A resin	2 mL	3 x 2 mL	+4°C
Spin Purification Columns	1 column	3 columns	+4°C
Binding Buffer (10X)	6 mL	18 mL	+4°C
Wash Buffer	25 mL	75 mL	+4°C
Elution Buffer	7 mL	21 mL	+4°C
Neutralizer	2 mL	6 mL	+4°C
Preservative	2 mL	6 mL	+4°C
Collection Tubes (15 mL)	4 tubes	12 tubes	+4°C
Spin Cartridge / Collecting Tube Assemblies	1 unit	3 units	+4°C

Reagents are ready to use as supplied.

3. Technical Hints

3.1 Preparation of tissue culture supernatant:

The antibody to be purified should be in 10 to 50 mL of tissue culture supernatant (TCS). Up to 5 mg of antibody can be purified in each run.

3.2 Compatibility with Nanoparticle Conjugation Kits

Antibodies purified using the TCS 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:

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

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 under-estimate of the IgG concentration.

For the 280 nm absorbance measurement, 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.

3.4 Protein A affinity for immunoglobulins:

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Pig	IgG	High
Mouse	IgG1	Low/Medium
Mouse	IgG2a	High
Mouse	IgG2b	High
Mouse	IgG3	Low/Medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low

4. Assay Procedure

– Equilibrate all materials and prepared reagents to desired purification temperature (4°C or RT) just prior to use and gently agitate.

4.1 Preparing tissue culture supernatant for binding:

Add the Binding Buffer (10X) to the tissue culture supernatant. The volume to add is 1/10 of the volume of tissue culture supernatant. For example, for 50 mL of tissue culture supernatant add 5 mL of Binding Buffer (10X). Mix by inversion.

4.2 Incubating sample with the resin:

Add the resin to the supernatant and use the supernatant to rinse the bottle to recover all the resin. 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 hrs.

4.3 Packing the column:

Carefully pour the supernatant-resin mix into the column. Sample volumes of more than 5 mL will have to be added in aliquots. The resin will collect at the bottom of the column. Snap off the bottom closure and place the column in a 15 mL collection tube.

Close the top with the screw-cap and spin at 1000 x *g* for 1 min. The unwanted supernatant will pass through the column and can be collected from the collection tube and kept on ice until a successful outcome has been confirmed. Alternatively, let the sample flow by gravity.

4.4 Wash procedure:

Wash the column with 3 mL of Wash Buffer to remove any non-bound protein. Cap the column and spin at 1000 x *g* for 1 min in the collection tube previously used.
Repeat the wash procedure three times. Alternatively wash by gravity with 5 mL of Wash Buffer three times.

*Δ **Note:** Wash the inner surface of the column to remove any residual starting material.*

4.5 Elution:

Please see Technical Consideration section on Test for protein before starting this step.

The antibody is eluted in 1 mL fractions. Aliquot 100 µL of Neutralizer in a clean collecting tube. Place the column in the collecting tube, add 1 mL of Elution Buffer and gently mix the resin by pipetting. Cap and spin at 1000 x *g* for 1 min to elute the protein.

Repeat the elution process two more times, using a clean collection tube each time.

Alternatively, add 1 mL Elution Buffer to the column and let flow by gravity in the tube containing 100 µL of Neutralizer.

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, by spin-purification, and in tubes 2 and 3 by gravity-purification, but you should confirm this using a test for protein before pooling any of the tubes.*

4.6 Antibody concentration (optional):

If the concentration of the recovered antibody is low then it can very quickly and easily be concentrated using the antibody concentrator Spin Cartridge / Collecting Tube Assemblies.

4.6.1 Add antibody to the top of the spin cartridge.

4.6.2 Spin for 1 to 3 mins* in a microfuge at a recommended maximum speed of 15,000 x *g* to reduce the buffer volume in the spin cartridge to between 50 and 100 µL.

4.6.3 Add more antibody to the spin cartridge, pipette to mix and spin as in Step 7.6.2. Repeat as many times as is necessary to process the entire antibody to the desired concentration. It may be necessary to discard any excess buffer collected in the collection tube between spins.

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

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

Δ Note: *Use a 1:10 dilution of Neutralizer in Elution buffer if you need to add buffer to your eluted antibody to maintain the right pH for antibody storage.*

Δ Note: ******* *Spin times will vary depending on buffer composition and volume as well as centrifuge speed.*

4.7 Addition of preservative (optional):

A bottle of Preservative is provided for optimal storage of your antibody and bacterial growth inhibition. The volume of preservative to add should be between 1/5 and 1/10 of the volume of your sample. The Preservative is compatible with our GOLD and Latex conjugation kits.

4.8 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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