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ab109207 TCS Antibody Purification Kit Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 862-0030, 862-0500

View ab109207
TCS Antibody Purification Kit datasheet:
www.abcam.com/ab109207
(use www.abcam.cn/ab109207 for China, or www.abcam.co.jp/ab109207 for Japan)

For the preparing IgGs for conjugation

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

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1. Overview

The TCS Antibody Purification kit has been designed for tissue culture supernatant purification and can be used to purify up to 50 mL in each purification.

This kit works by coupling highly purified protein A to agarose beads and can therefore be used to purify IgG fractions from hybridoma supernatants. The antibody is captured on the TCS resin and unwanted substances are removed by a simple wash procedure. The purified product is then eluted and neutralized.

The antibodies purified with the TCS Antibody Purification Kit are fully compatible with our Lightnign-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
	1 TEST	3 TESTS	
TCS Protein A resin	1 bottle	3 bottles	+4°C
Purification Columns	1 column	3 columns	+4°C
10x Binding Buffer	1 bottle	1 bottle	+4°C
Wash Buffer	1 bottle	1 bottle	+4°C
Elution Buffer	1 bottle	1 bottle	+4°C
Neutralization Buffer	1 bottle	1 bottle	+4°C
Concentrator Spin Columns	1 unit	3 units	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Amount of antibody that can be purified:

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 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 Protein A affinity for immunoglobulins:

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Pig	IgG	High
Mouse	IgG_1	Low/Medium
Mouse	IgG_{2a}	High
Mouse	IgG_{2b}	High
Mouse	IgG_3	Low/Medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low

3.4 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.

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 the rest of the fraction immediately as the low pH of the elution buffer can denature the antibody

***Δ 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 Preparing tissue culture supernatant for binding:

Add the 10x Binding Buffer to the tissue culture supernatant (add 1/10 of the volume of tissue culture supernatant). For example, for 50 mL of tissue culture supernatant add 5 mL of 10x Binding Buffer and mix by inversion.

4.2 Incubating sample with the resin:

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

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

4.3 Packing the column:

Support the column in an upright position and place a waste collection tube underneath (not provided). Carefully pour the supernatant-resin mix into the column. Sample volumes of more than 10 mL 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.

4.4 Wash procedure:

Wash the column with Wash Buffer to remove any non-bound protein. Place another waste collection tube (not provided) under the column and add 7 mL Wash Buffer to the top of the column. Wait until it has all passed through, and then repeat the wash procedure a total of three times. Keep the wash fractions again until a successful outcome has been confirmed.

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.

Elute the antibody in 1 mL fractions. Place a set of collection tubes under the column ready for elution. Add 1 mL of Elution Buffer to the column and collect the liquid.

Once all buffer has passed through the column, remove the collection tube from underneath the column and add 250 µL of Neutralization Buffer. Cap the tube, mix and place to one side.

Repeat the elution process three more times, each time neutralizing the sample as it is eluted.

Δ Note: The eluted antibody must be neutralized as soon as possible to avoid prolonged exposure to low pH of AbPure™ 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.

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

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 antibody to freeze thaw should be determined by experimentation on small aliquots.

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 | +86 21 2070 0500

Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829