ab128749
Mouse TCS Antibody Purification Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 832-0005, 832-0500.

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Mouse TCS Antibody Purification Kit datasheet:
www.abcam.com/ab128749
(use www.abcam.cn/ab128749 for China, or www.abcam.co.jp/ab128749 for Japan)

For preparing antibodies for conjugation.

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

Table of Contents

1. Overview

The Mouse TCS (Tissue Culture Supernatant) Antibody Purification kit has been designed for tissue culture supernatant purification.

Mouse resin has a very high affinity and specificity for mouse IgG molecules. ab128749 Mouse TCS Antibody Purification System can be used to purify mouse IgG fractions from hybridoma supernatants. The binding strength of bovine IgG to Abcam's Mouse resin is negligible; therefore, it can be used to selectively recover mouse IgG from TCS samples.

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

Antibodies purified using the Mouse 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 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	
Purification Column	1 unit	3 units	+4°C
Concentration Spin column and Collecting Tubes	1 unit	3 units	+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
Mouse resin	1 vial	3 vials	+4°C
Neutralization Buffer	1 vial	1 vial	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Recommended antibody quantities:

The mouse antibody to be purified should be in 10-25 mL of tissue culture supernatant. Up to 1.5 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 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.*

When using Bradford-type reagents it is important to use an IgG standard curve. Failure to do this will result in incorrect antibody levels being calculated. If IgG is not available then a BSA standard curve can be used, but the IgG levels will be under-estimated by a factor of 2.3.

4. Assay Procedure

4.1 Tissue culture supernatant Preparation:

Add the 10x Binding buffer to the tissue culture supernatant (add 1/10 of the volume of tissue culture supernatant). For example, for 20 mL of tissue culture supernatant add 2 mL of 10x Binding Buffer and mix by inversion.

4.2 Incubation of Sample with Resin:

Add the Mouse resin to the prepared 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 glass vial to recover all mouse resin.

4.3 Transfer of the Resin into 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 the Wash Buffer to remove any nonbound protein. Place another waste collection tube (not provided) under the column and add 7ml of the 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.

*Δ **Note:** Wash the inner surface of the column to remove any residual starting material. Keep the wash fraction until successful outcome is confirmed.*

4.5 Elution:

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

*Δ **Note:** 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 the buffer has passed through the column, remove the collection tube from underneath the column and add 250 µL of Neutrazlizing buffer and mix. Cap the tube and place to one side.

Repeat the elution process with a fresh collection tube three more times, each time neutralizing the sample as it is eluted.

*Δ **Note:** The Neutralizing buffer must be added to the sample as soon as possible to avoid prolonged exposure to low pH which can result in the denaturation of the IgG.*

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

4.6 Antibody Concentration (optional):

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

Add the mouse 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 mouse antibody to the desired concentration. It may be necessary to discard any excess buffer collected in the tubes between spins.

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

*Δ **Note:** It is advisable not to spin the mouse 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 mouse antibody to freeze-thaw should be determined by experimentation on small aliquots.

Version 5b

Technical Support

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