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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Anti-mCherry Antibody Sepharose Purification Kit

Catalogue Number LDG0022RD

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO

Store at 2-8℃

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Leadgene® Anti-mCherry Antibody Sepharose Purification Kit

1. Introduction

Anti-mCherry sepharose, is consist of an mCherry VHH coupled to NHS-sepharose. It is an efficient technique for isolating recombinant proteins or mammalian expression proteins. The purified antibody is immobilized at 2-4 mg antibody per ml of 50% slurry and this kit allows a rapid and efficient affinity purification of active mCherry fusion proteins.

2. Test principle

The mCherry epitope system relies on a VHH mCherry recombinant antibody, which is able to react with N- and C-terminal RFP, DsRed and mCherry tagged fusion proteins. This product can be used for the immunoprecipitation or immune affinity purification. The affinity resin allows an efficient binding of mCherry fusion proteins without the need for preliminary steps and calibrations. The affinity bound mCherry fusion proteins can be efficiently eluted from the resin by acid condition. The eluted proteins can be used for characteristic analysis.

3. Reagents provided and reconstitution

Reagents (Store at 2-8°C)	Quantity for 5 rxns (LMRD022005C040)	Quantity for 10 rxns (LMRD022010C040)	Composition	Reconstitution
Anti-mCherry sepharose	1 vial (1 mL)	1 vial (2 mL)	50% slurry of Anti-mCherry sepharose in in 20% ethanol	The sepharose must be washed before use and should be equilibrated with 1X Wash Buffer.
Wash Buffer (10X concentration)	1 vial (2.5 mL)	1 vial (5 mL)	100 mM Tris/Cl pH 7.5; 1.5 M NaCl; 5 mM EDTA	Dilute to 1X working concentration with distilled water.
Elution Buffer	1 vial (5 mL)	1 vial (10 mL)	200 mM Glycine pH 2.5	Ready for use
Neutralization Buffer	1 vial (1 mL)	1 vial (2 mL)	2 M Tris pH 8.0	Ready for use
spin column	5 pcs	10 pcs	-	-
collection tube	5 tubes	10 tubes	-	-

4. Materials required but not provided

(1) High quality distilled water

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(2) 5 μL to 1000 μL adjustable single-channel micropipettes with disposable tips
(3) Micro-centrifuge capable of 15,000 x g
(4) 1.5 mL microcentrifuge tubes
(5) CoIP Lysis Buffer (mild reaction): 10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5mM EDTA; 0.5% NP-40
(6) RIPA (vigorous reaction): 100 mM Tris/Cl pH 7.5; 300 mM NaCl; 0.2% Sodium Deoxycholate (or 0.1% SDS); 2% NP-40
(7) Beakers, flasks, cylinders necessary for preparation of reagents
(8) 2 x SDS Loading Buffer without DTT & 2 x SDS Loading Buffer with 1 mM DTT
(9) Pre-urea Buffer: 50 mM Tris pH 8.5; 1mM EGTA; 75 mM KCl
(10) Urea Elution Buffer: 6-8 M urea; 20 mM Tris/Cl pH 7.5; 100 mM NaCl.
(11) Timer
(12) Clean paper towels
(13) Disposable gloves
(14) Discard container for bio-medical waste

5. Reagent preparation

- **Working wash buffer (1 X):** Dilute 1 volume of **10 X wash buffer** with 9 volumes of distilled water and homogenize by using micropipette.

6. Storage of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at 2-8℃. Please see the box front label for expiration date.
- For sustainable use and long term storage, store at 2 °C to 8 °C. **DO NOT FREEZE.**
- All working reagents should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.
- To minimize protein degradation, protease inhibitor cocktails are highly recommended.
- For best results, determine optimal conditions for expression of mCherry fusion protein before attempting immunoprecipitation.

7. Precautions & warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those persons who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.

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- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- Do not mix or substitute reagents with those from different lots or other sources.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Use disposable graduated pipettes and tips to avoid cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

8. Procedure

- The Anti-mCherry sepharose is stored in 20% ethanol. The sepharose must be washed before use and should be equilibrated with 1X Wash Buffer. The equilibration can be performed at room temperature or at 2-8 °C.
- In the case of bulk reaction. Users can make a pre-reaction through mixing sample and sepharose in 15 mL / 50 mL tube, and then transfer the mixture into the column for binding.
- The binding and elution capacity of 1 mL settled Anti-mCherry sepharose may be vary, commonly more than 1 mg RFP, DsRed or mCherry fusion proteins. Trying different elution buffers for optimal results is recommended.
- Cellular debris and particulate matter must be removed by centrifugation or filtration prior to purification on the column.
- Highly viscous samples which may contain chromosomal DNA or RNA should be sonicated or treated with nuclease to decrease the viscosity.
- Perform all steps on ice.

Purification of mCherry fusion proteins

- **Sample preparation (Lysis of Mammalian Cells)**

(1) Detach the cells from the culture dish and collect the cell suspension into the centrifuge tube.
(2) Centrifuge the cell suspension at 400 x g for 5 minutes to pellet the cells. Carefully remove and discard the supernatant.
(3) Wash cells by re-suspending the cell pellet in ice-cold PBS.
(4) Centrifuge the cell suspension at 400 x g for 5 minutes to pellet the cells. Carefully remove and discard the supernatant.
(5) Add 200 µL of Lysis buffer to the cell pellet and vortex.
(6) Incubate the sample for 15 minutes on ice.
(7) Remove cell debris by centrifugation at 15,000 x g for 5 minutes at 4°C.

- **Column preparation**

(1) Place an empty spin column on the collection tube.
(2) Wash the column with 200 µL Wash Buffer.
(3) Allow the buffer to drain from the column and leave residual Wash Buffer in the column to aid in packing the Mouse anti- mCherry sepharose, then discard the buffer in the collection tube.

- **Packing the Column**

- (Note: Make sure the column filter is fixed in the correct position before transferring the sepharose).

(1) Completely suspend the vial of Mouse anti- mCherry sepharose.

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(2) Transfer 200 µL volume to an empty centrifuge tube and wash the sepharose with 1 mL Wash Buffer.
(3) Spin down the sepharose with 100 x g, for 30 seconds and discard supernatant.
(4) Immediately transfer the sepharose to the spin column. Allow the sepharose bed to settle. Please prevent the sepharose bed from getting dried.

- **Binding mCherry fusion protein to the column**

(1) Dilute the sample with Wash Buffer in 1:3 proportion.
(2) Load the sample on the spin column and centrifuge the column at 100 x g for 30 seconds. Users can also perform this binding reaction in a new 1.5 mL centrifuge tube.
- (Note: Depending upon the mCherry fusion protein and the flow rate, not all of the protein may bind. Repeat loading the sample to increase binding efficiency).
(3) Collect the fractions using empty centrifuge tube.
(4) Wash the spin column with 300 µL Wash Buffer more than 6 times.
- (Note: To eliminate the noisy band in sample, more washing step is recommended).

- **Elution of mCherry fusion protein**

(1) Add 5 x 100 µL Elution Buffer to elute the bound mCherry fusion protein from the spin column to the collection tube. This step can be supported by a centrifugation at 100 x g for 30 seconds.
(2) Immediately neutralize the eluted sample by adding 10 µL Neutralization Buffer. Assay sample concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance.
- (Note: Measuring the absorbance after each Elution move can help collecting the sample more accurately).

- **Option I instead of elution step**

(1) Elute 50 µL of Anti-mCherry sepharose by heating in 50 µL of 2 x SDS Loading Buffer without DTT for 10 min at 50°C.
(2) Pellet sepharose, transfer supernatant to a new tube and add DTT at 1 mM (elution 1).
(3) Add 50 µL 2 x SDS Loading Buffer with 1 mM DTT to pelleted beads (elution 2).
(4) Boil the eluted samples for 5 min and analyze content of the sample by western blot. Generally, there should be target protein in both elution 1 and 2 although the quantity in each will be variable and elution 2 will have more IgG contamination than elution 1.

- **Option II instead of elution step**

(1) Wash Anti-mCherry sepharose with Pre-urea Buffer. Remove all residual supernatant.
(2) Add 100-250 µL Urea Elution Buffer and rotate for 30 min at room temperature with frequent agitation before gentle centrifugation.
(3) Repeat this process at least twice more to ensure that the entire captured complex has been released from the beads. Pellet beads and remove urea to a new tube.
(4) Run the samples on a western blot to check the precipitation of proteins.

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