PRODUCT INFORMATION

Anti-EGFP sepharose Purification kit

For mammalian expression system

v. 230201

Catalog number	C07009-K01 / C07009-K02
Package	5 rxns / 10 rxns
Description	Anti-EGFP sepharose, is consist of an EGFP V_HH coupled to NHS-sepharose. It is an efficient technique for isolating recombinant proteins or mammalian expression proteins. The EGFP epitope system relies on a V_HH EGFP recombinant antibody, which is able to react with N- and C- terminal GFP tagged and EGFP tagged fusion proteins. This product can be used for the immuneprecipitation or immune affinity purification. The purified antibody is immobilized at 3-5 mg antibody per mL of 50% slurry and this kit allows a rapid and efficient affinity purification of active EGFP fusion proteins. The affinity resin allows an efficient binding of EGFP fusion proteins without the need for preliminary steps and calibrations. The affinity bound EGFP fusion proteins can be efficiently eluted from the resin by acid condition. The eluted proteins can be used for characteristic analysis.

Component

Reagents & Materials	Quantity for 10 rxns (C07009-K02)	Quantity for 5 rxns (C07009-K01)	composition
Anti-EGFP sepharose	2 mL X 1 vial	1 mL X 1 vial	50% slurry of Anti-EGFP sepharose in 20% ethanol
Wash Buffer (10X concentration)	5.0 mL X 1 vial	2.5 mL X 1 vial	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Elution Buffer	10 mL X 1 vial	5 mL X 1 vial	200 mM Glycine pH 2.5
Neutralization Buffer	2 mL X 1 vial	1 mL X 1 vial	2 M Tris pH 8.0
spin column	10 pcs	5 pcs	
collection tube	10 tubes	5 tubes	

Note: Sepharose is 1:1 suspension in 20% ethanol as a preservative.

Product capacity	The binding and elution capacity of 1 mL settled Anti-EGFP sepharose may be vary, commonly more than 1 mg GFP or EGFP fusion proteins. Trying different elution buffers for optimal results is recommended.
Materials Required but Not Provided	• Micro-centrifuge capable of 15,000 x g • 1.5 mL Centrifuge tubes

	• End-over-end rotator • CoIP Lysis Buffer (mild reaction): 10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40 • RIPA (vigorous reaction): 100 mM Tris/Cl pH 7.5; 300 mM NaCl; 0.2% Sodium Deoxycholate (or 0.1% SDS); 2% NP-40 • 2 x SDS Loading Buffer without DTT & 2 x SDS Loading Buffer with 1 mM DTT • Pre-urea Buffer: 50 mM Tris pH 8.5; 1mM EGTA; 75 mM KCl • Urea Elution Buffer: 6-8 M urea; 20 mM Tris pH 7.5; 100 mM NaCl.
Storage	For sustainable use and long term storage, store at 2 °C to 8 °C. **DO NOT FREEZE.**
Precautions and Disclaimer	This product is for R&D use only, not for drug, household, or other uses.
Procedure	<u>General notes</u> The Anti-EGFP 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. The Wash Buffer is original stock concentration (10X). Dilute Wash Buffer to 1X working concentration with distilled water. 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. <u>Suggestions on purification of EGFP fusion protein</u> 1. Cellular debris and particulate matter must be removed by centrifugation or filtration prior to purification on the column. 2. Highly viscous samples which may contain chromosomal DNA or RNA should be sonicated or treated with nuclease to decrease the viscosity. 3. Perform all steps on ice. A. 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.

Procedure	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. B. 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 Anti-EGFP sepharose, then discard the buffer in the collection tube. C. Packing the column 1. Completely suspend the vial of Anti-EGFP sepharose. 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, 30 seconds' centrifugation 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. (Note: Make sure the column filter is fixed in the correct position before transferring the sepharose). D. Binding EGFP 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 EGFP 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)

E. Elution of EGFP fusion protein

1. Add 5 x 100 µL Elution Buffer to elute the bound EGFP 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 step can help collecting the sample more accurately).

F. Option I instead of elution step

1. Elute 50 µL of Anti-EGFP 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.

G. Option II instead of elution step

1. Wash Anti-EGFP sepharose with Pre-urea Buffer (mentioned in material not provided). Remove all residual supernatant.
2. Add 100-250 µL Urea Elution Buffer (mentioned in material not provided) 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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