PRODUCT INFORMATION

Protein A sepharose Purification kit

For mammalian expression system

v. 230201

Catalog number	C07005-K01 / C07005-K02
Package	5 rxns / 10 rxns
Description	Affinity purified Protein A antibodies, developed in mouse, were conjugated to NHS-sepharose. Protein A is a 42 kDa surface protein found in the cell wall of *Staphylococcus aureus*. Protein A has a high affinity to Human IgG, Mouse IgG and others and is therefore suitable for purification and detection of immunoglobulins. Therefore, Croyez Protein A sepharose is an efficient technique for IgG purification. Recombinant protein A is immobilized at over 5 mg antibody per mL 50% slurry and this kit allows a rapid and efficient affinity purification of active IgG proteins. The affinity resin allows an efficient binding of IgG proteins without the need for preliminary steps and calibrations. The affinity bound IgG 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 (C07005-K02)	Quantity for 5 rxns (C07005-K01)	composition
Protein A sepharose	2 mL X 1 vial	1 mL X 1 vial	50% slurry of Protein A sepharose in 1X Phosphate Buffered Saline
Wash Buffer (10X concentration)	5.0 mL X 1 vial	2.5 mL X 1 vial	100 mM Tris/Cl pH 7.5; 1.5 M NaCl; 5 mM EDTA
Elution Buffer	10 mL X 1 vial	5 mL X 1 vial	0.1 M Glycine pH 2.7
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 Phosphate Buffered Saline, pH 7.4, containing 0.05% sodium azide as a preservative.

Product capacity	The binding and elution capacity of 1 mL settled Protein A sepharose are commonly more than 1 mg of IgG 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
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 Protein A sepharose is stored in Phosphate Buffered Saline containing 0.05% sodium azide. The PBS must be removed before use and the resin 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. Dilute Wash Buffer (10X concentration) 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 IgG 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 containing 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. 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 Protein A sepharose, then discard the buffer in the collection tube.

C. Packing the column

1. Completely suspend the vial of Protein A 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).

Procedure

D. Binding IgG protein to the column

1. Dilute the sample with Wash Buffer in 1:3 proportion.
2. Load the sample on the 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 Eppendorf tube.
   (Note: Depending upon the IgG 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 IgG protein

1. Add 5 x 100 µL Elution Buffer to elute the bound IgG 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 (1 OD_{280} = 0.73 mg/mL IgG).
   (Note: Measuring the absorbance after each Elution move can help collecting the sample more accurately).

F. Optional instead of elution step

Resuspended Protein A sepharose in 100 µL 2 x SDS-Sample Buffer for 10 minutes at 95°C to dissociate immune-complexes from Protein A sepharose. Protein A sepharose can be collected by centrifugation at 2500 x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant.

For Research Use Only.