Protein G Antibody Purification Kit

P1455933

Storage 2-8℃.

Shipping Transport with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

Streptococcal protein G, abbreviated as Protein G, is a cell wall protein derived from streptococci, with a molecular weight of approximately 25 kDa. It consists of albumin-binding domains (ABD1, ABD2, ABD3) and immunoglobulin-binding domains. Similar to Protein A, Protein G can specifically bind to the Fc region of IgG; however, Protein G exhibits a broader spectrum of antibody-binding specificity, with superior binding capacity for IgG from non-common species, lower serum protein binding levels, and higher purity.Protein A and Protein G differ in their binding properties. Compared with Protein A, Protein G shows stronger affinity for polyclonal antibodies from cattle, sheep, horses and other species. It can also bind to rat IgG, human IgG3 and mouse IgG1-antibody subtypes that cannot be effectively bound by Protein A.When used as an affinity ligand and conjugated to a Sepharose matrix, Protein G can specifically bind to antibody molecules in samples while allowing other impurity proteins to flow through, achieving extremely high selectivity. A single step of affinity chromatography can yield antibody purity of over 95%.

This kit is capable of purifying antibodies from various sample types, including ascites, serum, and cell culture supernatants. The Protein G purification filler in the kit can be reused more than 10 times. Protein G conjugated to Sepharose beads serves as an ideal choice for purifying monoclonal or polyclonal IgG antibodies from humans, rats, mice, goats and sheep, featuring high specificity, high stability, high affinity, and low ligand leaching.

Product components

P1455933	Components	2 mL	10 mL	Storage	Quantity Per sample
P1455933A	Binding buffer(10×)	50 mL	250 mL	2-8℃	5 mL
P1455933B	Elution buffer(5×)	10 mL	50 mL	2-8℃	1 mL
P1455933C	Neutralizer Buffer	10 mL	50 mL	2-8℃	1 mL
P1455933D	Protein G resin	2 mL	10 mL	2-8℃	2 mL
P1455933E	Purification Column	1 EA	5*1 EA	2-8℃	1 EA
P1455933F	Coomassie Brilliant Blue Staining Solution	10 mL	50 mL	2-8℃	1 mL
P1455933G	Regeneration Buffer	10 mL	50 mL	2-8℃	10 mL (Optional)

Note: Each plate is sufficient for one 96-well microplate.

Instructions for Use

Prior to use, dilute all component buffers to 1× concentration with deionized water. Specific operations can be adjusted according to the requirements of different purification tools.

1. Sample Preparation

Before purification, clarify hybridoma supernatants, serum, or ascites samples by centrifugation or filtration through a 0.45 μm filter to avoid clogging the purification column and reducing its lifespan.

Note: The dynamic binding capacity (DBC) of the Protein G resin provided in this kit is ≥30 mg hIgG/mL. To avoid overloading the resin and ensure purification efficiency, it is recommended that the actual IgG loading amount should not exceed 80% of this DBC (i.e., the total IgG load per column per run should be ≤24 mg). Due to variations in the expression characteristics of hybridoma cell clones, the antibody concentration in the tissue culture supernatant can fluctuate significantly. Therefore, the typical yield per purification run generally ranges between 2 mg and 10 mg. The specific yield should be determined based on actual experimental conditions, such as the antibody concentration in the supernatant and the sample volume.

2. Sample Purification

2.1 Column Packing

a. Fix the purification column vertically and ensure the bottom frit is securely attached horizontally.

b. Add 2 mL of Protein G Resin to the column tube. After the resin settles at the bottom, carefully insert the top frit and compact the resin gently.

c. Remove the bottom cap and allow the liquid in the column to drain naturally. The final effective volume of the Protein A purification column is 1 mL.

Notes: The Protein G Resin contains 50% protective solution; mix thoroughly before packing to avoid affecting purification efficiency.When placing the frit, maintain a flat liquid level in the column to prevent air bubbles between the resin and the frit. Bubbles will reduce separation efficiency and increase the pressure required to maintain a stable flow rate.

2.2 Equilibration

Fill the purification column with 1× Binding Buffer (approximately 15 column volumes) to equilibrate the column, allowing the liquid to drain naturally.

2.3 Sample Loading

Remove the top cap of the purification column and add the clarified sample to the Protein G purification column, allowing the liquid to drain naturally.

Note: If the antibody concentration in the sample is low or the sample volume is large, increase the number of loading cycles or extend the loading time to improve the binding efficiency between the antibody and the resin.

2.4 Washing

Fill the purification column with 1× Binding Buffer (approximately 15 column volumes) to wash away unbound impurities, allowing the liquid to drain naturally.

2.5 Elution

a. Prepare several 1.5 mL centrifuge tubes and pre-add 10 μL of Neutralizer Buffer to each

tube.

b. Add 3 mL of 1× Elution Buffer (approximately 3 column volumes) to each purification column and collect the eluate by gravity. Collect 8 drops (approximately 0.5 mL) per tube and repeat until elution is complete.

2.6 Dialysis

a. Take 2.5 μL of eluate from each tube, mix with 10 μL of Coomassie Brilliant Blue Staining Solution, and observe the gradient change of antibody elution.

b. Collect the eluate tubes with high antibody concentration and dialyze against an appropriate buffer to obtain highly purified IgG.

3. Storage of Purification Column

a. After antibody elution, add an additional 2 mL of 1× Elution Buffer to the column to wash away any residual antibody.

b. Fill the purification column with 1× Binding Buffer (approximately 15 column volumes) to equilibrate the column, allowing the liquid to drain naturally.

c. Fill the purification column with 20% ethanol (approximately 15 column volumes) for preservation, allowing the liquid to drain naturally.

d. Add 5 mL of 20% ethanol to the column, cap the top, and seal the bottom cap. Store at 2-8℃ for direct use in subsequent purifications.

4. Column Regeneration (Optional)

Perform regeneration when a measurable decrease in column performance is observed, following these steps:

a. Add 10 mL of Regeneration Buffer to the column for washing.

b. Add 30 mL of double-distilled water (ddH_2O) to rinse the column thoroughly and remove residual Regeneration Buffer.

c. Add 20 mL of 1× Binding Buffer to wash and equilibrate the resin.

d. Add 15 mL of 20% ethanol for washing, then add 5 mL of 20% ethanol to the column. Cap the top and seal the bottom cap, and store at 2-8℃