Protein A Antibody Purification Kit

P1455932

Storage 2-8℃.

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

Introduction

Protein A is a protein with a molecular weight of approximately 42 kDa, consisting of a single polypeptide chain without disulfide bonds. Its structure contains multiple highly homologous IgG-binding domains (typically 4-5), each composed of about 55-62 amino acid residues. This protein exhibits remarkable stability, retaining activity even when exposed to urea, acidic conditions (pH 2.5), or boiling. The binding range covers IgG from various mammals (e.g., humans, pigs, rabbits, mice), but the binding capacity varies among different species and IgG subclasses. For example, human IgG1, IgG2, and IgG4 subclasses form stable complexes with Protein A, while IgG3 lacks binding ability due to key site mutations in the Fc fragment; murine IgG1 shows significantly lower binding affinity to Protein A compared to subclasses such as IgG2a and IgG2b. Despite these binding differences, Protein A remains one of the most widely used ligands in antibody affinity purification, thanks to its high specific binding to IgG from most commonly used model organisms.

The Protein A Antibody Purification Kit is a standardized experimental tool designed for routine antibody purification, enabling efficient and reproducible isolation of IgG. The kit works by conjugating high-purity Protein A to highly cross-linked Polystyrene-Divinylbenzene (PS-DVB) microspheres and is suitable for purifying IgG fractions from hybridoma cell supernatants.

Product Components

P1455932	Component	20 T	50 T	Storage	Quantity Per Test
P1455932A	Binding buffer(10×)	50 mL	250 mL	2-8℃	5 mL
P1455932B	Elution buffer(5×)	10 mL	50 mL	2-8℃	1 mL
P1455932C	Neutralizer Buffer	10 mL	50 mL	2-8℃	1 mL
P1455932D	Protein A resin	2 mL	10 mL	2-8℃	2 mL
P1455932E	Purification Column	1 unit	5 unit	RT or 2-8℃	1 unit
P1455932F	Coomassie Brilliant Blue Staining Solution	10 mL	50 mL	2-8℃	1 mL
P1455932G	Regeneration Buffer	10 mL	50 mL	2-8℃	10mL (Optional)

Note: The recommended amount of Protein A resin for each test is 2 mL.

Instruction 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.

I. 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 A 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.

II. Sample Purification

1. Column Packing

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

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

1.3 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 A 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. Equilibration

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

3. Sample Loading

Remove the top cap of the purification column and add the clarified sample to the Protein A 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.

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.

5. Elution

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

5.2 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.

6. Dialysis

6.1 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.

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

III. Storage of Purification Column

1. After antibody elution, add an additional 2 mL of 1× Elution Buffer to the column to wash away any residual antibody.
2. Fill the purification column with 1× Binding Buffer (approximately 15 column volumes) to equilibrate the column, allowing the liquid to drain naturally.
3. Fill the purification column with 20% ethanol (approximately 15 column volumes) for preservation, allowing the liquid to drain naturally.
4. 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.

IV. Column Regeneration (Optional)

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

1. Add 10 mL of Regeneration Buffer to the column for washing.
2. Add 30 mL of double-distilled water (ddH_2O) to rinse the column thoroughly and remove residual Regeneration Buffer.
3. Add 20 mL of 1× Binding Buffer to wash and equilibrate the resin.
4. 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℃.