Protein L Plus Agarose Gel, 30-100 µm
PRODUCT DATA SHEET

Protein L Plus Agarose Gel, 30-100 µm

Cat No: AG-Protein L-3000

Description

Protein L Plus agarose gel is a self-made agarose gel with recombinant Protein L protein as the ligand. It has high physical and chemical stability, and the ligand is not easy to fall off. It has long life and is easy to use. Recombinant Protein L is an immunoglobulin binding protein that binds to the light chain of immunoglobulin K without affecting antigen binding. Compared with other antibody-binding proteins such as Protein A and Protein G, Protein L has a wider binding range of Ig and Ig subtypes.

The product has higher antibody binding capacity and lower non-specific protein adsorption rate. The elution conditions are uniform, and the purity of antibodies greater than 90% can be separated from serum samples in one step. This product is suitable for antibody purification in plasma, ascites, tissue culture supernatant and other samples, and can also be used for immunoprecipitation and other related studies.

Characteristics

Matrix	4% highly crosslinked agarose gel (4FF)
Ligand	Recombinant Protein L protein
Mean particle size	30~100 µm
Concentration	The gel volume accounts for 50% of the suspension volume
Antibody binding capacity	> 20 mg Human IgG/mL Gel
Withstand voltage	0.5 MPa
pH stability	3~12 (working) 2~14 (CIP)
Chemical stability	Common aqueous solution: 10 mM HCl, 0.1 M citric acid (pH 3), 6 M urea, 6 M guanidine hydrochloride, 30% isopropyl alcohol, 20% ethanol
Shelf life	Stable storage at 2 ~ 8°C for two years

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Operation process

(Take purified human serum IgG as an example)

Recommended buffer:

Binding/Washing buffer	0.5 M NaCl, 20 mM Na_2HPO_4, pH 7.0
Elution buffer	100 mM Gly, pH 3.0
Neutrilization buffer	1.0 M Tris-HCl, pH 8.5

1. Sample preparation

Before submitting the sample, ensure that the sample solution has the appropriate ionic strength and pH value, dilute the serum sample, ascites, or cell culture solution with **Binding/Washing buffer**, or place the sample on **Binding/Washing buffer** dialysis. It is recommended that the samples be centrifuged and filtered with 0.22 μm or 0.45 μm filter membrane before loading to reduce impurities and improve the efficiency of protein purification.

2. Gel filling

1) Take the appropriate size of the affinity chromatographic column, put it into the gasket, add an appropriate amount of deionized water washing column tube and gasket, and close the lower outlet.

2) **Protein L Plus agarose gel** was evenly mixed, appropriate amount of slurry was absorbed with a pipette and added to the chromatographic column (the actual volume of gel accounted for half of the suspension), and the lower outlet was opened to discharge the protective fluid.

3) Add appropriate amount of deionized water to rinse the gel, and close the lower outlet when the liquid in the column flows out.

4) The loaded chromatographic column should be added into **Binding/Washing buffer** for balance. If not used for the time being, protective solution should be added and stored at 2 ~ 8°C.

3. Sample purification

3.1 Purification by incubation

1) According to the purified sample size, appropriate amount of **Protein L Plus agarose gel** was added into the centrifuge tube, centrifuged at 1000 rpm for 1 min, and the supernatant was removed;

2) Add 5 times the **Binding/Washing buffer** into the centrifuge tube to clean the gel, centrifuge at 1000 rpm for 1 min, remove the supernatant, and repeat for more than 2 times.

3) The samples were added and incubated at 2~8°C for 2~ 4 h or overnight.

4) After incubation, centrifuge at 1000 rpm for 1 min, absorb the supernatant to the new centrifuge tube, and retain the supernatant as a flow for electrophoresis identification.

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5) Wash the gel with a **Binding/Washing buffer** that is 5 times the gel volume, centrifuge at 1000 rpm for 1 min, remove the supernatant (be careful not to inhale the gel), and repeat for 3 to 5 times, with a new centrifuge tube recommended for washing.

6) **Elution buffer** of 3 ~ 5 times gel volume was added for elution, incubated at room temperature for 5~15 min, centrifuged at 1000 rpm for 1 min to collect eluent, which could be repeated 2 ~ 3 times. The eluent needs to be neutralized immediately, and a **Neutrilization buffer** of 1/10 of the elution component volume is generally recommended for neutralization.

3.2 Purification by chromatographic column

The amount of solution is calculated by the column volume (for example, 5 times the column volume, 1 mL specification corresponds to 5 mL solution, 10 mL specification corresponds to 50 mL solution).

1) The loaded **Protein L Plus agarose gel** chromatographic column was balanced with a **Binding/Washing buffer** of 5 times the column volume, so that the gel was placed under the same buffer liquid system as the target protein, and repeated 2 ~ 3 times.

2) The sample was added to the balanced chromatographic column and incubated in the rotary mixing apparatus for 30 ~ 60 min, then the effluent was collected, and the sample could be repeated to increase the binding efficiency.

3) A **Binding/Washing buffer** of 10 ~ 15 times the column volume was used to wash impurities, remove the impurities unless specifically adsorbed, and collect the washing solution.

4) **Elution buffer** of 5 ~ 10 times column volume was used to collect the elution buffer in stages, one tube was collected for each column volume, and the antibody with high purity and high concentration could be obtained to ensure that all bound target proteins were elated. The elution component needs to be neutralized immediately, and a **Neutrilization buffer** of 1/10 of the elution component volume is generally recommended for neutralization.

3.3 SDS-PAGE detection

Samples obtained from purified products (including flow through components, wash components, and elution components) and original samples were tested using SDS-PAGE.

4. Gel wash

Protein L Plus agarose gel can be reused without regeneration, but with the precipitation of some denaturing substances and the aggregation of proteins, the flow rate and binding load are often reduced, seriously affecting the performance of the column, at which time the gel needs to be cleaned.

Remove some precipitated or denatured material

Clean with 2 times the column volume of 6M guanidine hydrochloride solution, then immediately clean with 5 times the column volume of PBS (pH 7.4).

Remove some non-specific adsorbent substances caused by hydrophobic adsorption

Clean with 70% ethanol of 3 ~ 4 times column volume or 1% Triton X-100 of 2 times column volume, then immediately clean with 5 times column volume of PBS (pH 7.4).

Notes

1. Before performing the experiment, please read this operation manual carefully.
2. Agarose gel should be thoroughly mixed before use. The gel should be kept in storage solution to prevent drying.
3. This product is for scientific research only.

Ordering Information

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