Protein G Agarose

Description

TargetMol Protein G Agarose consists of recombinant Protein G immobilized at high density and in an oriented manner on highly cross-linked agarose beads. Recombinant Protein G exhibits strong binding affinity for mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c, rabbit and goat polyclonal antibodies, as well as human IgG1, IgG2, IgG3, and IgG4.

TargetMol Protein G Agarose is suitable for the isolation and purification of IgG from samples such as cell lysates, cell culture supernatants, serum, and ascites fluid.

Features

- Good physicochemical stability
- Low ligand leakage
- High durability
- Easy to use

Product Information

Property	Specification
Matrix	Highly cross-linked 4% agarose
Particle Size	30–100 μm
Ligand	Recombinant Protein G
Binding Capacity	≥15 mg hIgG/mL gel
Concentration	25% (v/v)

Applications

- Isolation and purification of IgG from cell lysates, cell culture supernatants, serum, ascites fluid, and related samples.
- Immunoprecipitation (IP), co-immunoprecipitation (Co-IP), chromatin immunoprecipitation (ChIP), and RNA immunoprecipitation (RIP) of proteins and protein complexes.

Instructions

1. Buffer Preparation

The following buffer formulations are commonly used. It is recommended to filter all buffers through a 0.22 μm or 0.45 μm membrane before use.

1) Equilibration/Wash Buffer: 0.15 M NaCl, 20 mM Na2HPO4, pH7.0
2) Acidic Elution Buffer: 0.1 M Glycine, pH3.0
3) Neutralization Buffer: 1 M Tris-HCl, pH8.0

2. Sample Preparation

1) Before sample loading, ensure that the sample solution has an appropriate ionic strength and pH. Serum samples, ascites fluid, or cell culture supernatants can be diluted with equilibration/wash buffer, or equilibrated against the equilibration/wash buffer by dialysis.
2) To reduce impurities, improve purification efficiency, and prevent column clogging, samples should be centrifuged or filtered through a 0.22 μm or 0.45 μm membrane before loading.

3. Agarose Packing: Packing a gravity-flow column

1) Select an appropriately sized gravity-flow chromatography column, install the bottom frit, add an appropriate amount of purified water to rinse the column and frit, and then close the outlet.
2) Thoroughly resuspend the Protein G agarose and transfer an appropriate volume of slurry into the gravity-flow column using a pipette tip (the settled gel volume accounts for approximately one-half of the slurry volume). Open the outlet and allow the storage solution to drain completely.
3) Add an appropriate amount of purified water to wash the agarose. After the liquid has drained completely by gravity, close the outlet.
4) Install the pre-wetted top frit, ensuring that there is no gap between the frit and the agarose bed and that the frit remains level.
5) The packed gravity-flow column may be equilibrated immediately with equilibration buffer. If not used immediately, add storage solution and store at 2–8°C.

4. Sample Purification

A. Gravity-Flow Column Purification

1) Equilibrate the packed Protein G agarose gravity-flow column with 5 column volumes (CV) of equilibration buffer. Repeat 2–3 times to ensure that the agarose is in the same buffer system as the target protein.
2) Load the sample onto the equilibrated column and allow it to remain in the column for at least 2 min to ensure sufficient contact with the agarose. Collect the flow-through. The sample may be reloaded multiple times to increase binding efficiency.
3) Wash the column with 10–15 CV of wash buffer to remove non-specifically bound proteins and collect the wash fractions.
4) Elute the bound protein with 5–10 CV of acidic elution buffer. Collect the eluate in fractions, typically one fraction per column volume, to monitor elution efficiency and ensure complete recovery of the target protein while maintaining high purity and concentration. The eluate should be neutralized immediately. Typically, 1/10 volume of neutralization buffer is recommended.

Note: After elution, wash the column sequentially with 3 CV of equilibration buffer, 5 CV of purified water, and 2 CV of 20% ethanol. Store the agarose at 2–8°C.

B. Batch Purification Method

1) According to the amount of sample to be purified, thoroughly resuspend the Protein G agarose and transfer an appropriate amount into a centrifuge tube. Centrifuge at 1000 rpm for 5 min and discard the supernatant. Alternatively, load the agarose into a gravity-flow column and allow the storage solution to drain completely.
2) Add 5 gel volumes of equilibration buffer to wash the agarose. Centrifuge at 1000 rpm for 5 min and discard the supernatant. If using a gravity-flow column, wash directly in the column and allow the equilibration buffer to drain completely. Repeat this step at least twice.
3) Add the sample and seal the centrifuge tube or gravity-flow column. Incubate with shaking at 4°C for 2–4 h, or incubate at 37°C for 30 min–2 h.

4) After incubation, centrifuge at 1000 rpm for 5 min and discard the supernatant, or collect the agarose by filtration. Retain the supernatant as the flow-through fraction for SDS-PAGE analysis.
5) Wash the agarose with 5 gel volumes of wash buffer. Centrifuge at 1000 rpm for 5 min, or remove the supernatant using a gravity-flow column (avoid aspirating the agarose). Repeat 3–5 times. It is recommended to transfer the agarose to a fresh centrifuge tube during the washing procedure.
6) Add 3–5 column volumes of acidic elution buffer and incubate at room temperature for 5 min. Centrifuge at 1000 rpm for 5 min or collect the eluate using a gravity-flow column. The elution step may be repeated 2–3 times. The eluate should be neutralized immediately. Typically, 1/10 volume of neutralization buffer is recommended.

5. SDS-PAGE Analysis

Analyze the original sample, flow-through fractions, wash fractions, and elution fractions by SDS-PAGE to evaluate the purification performance.

6. Agarose Cleaning

Protein G agarose may be reused without regeneration. However, the accumulation of non-specifically bound proteins and protein aggregates may lead to reduced flow rate and binding capacity. In such cases, the agarose can be cleaned as follows:

1) Removal of Precipitates or Denatured Materials

Wash with 2 column volumes of 6 M guanidine hydrochloride solution, followed immediately by 5 column volumes of PBS (pH 7.4).

2) Removal of Non-Specifically Bound Hydrophobic Contaminants

Wash with either 3–4 column volumes of 70% ethanol, or 2 column volumes of 1% Triton X-100. Then immediately wash with 5 column volumes of PBS (pH 7.4).

Storage Conditions

Store at 4°C. Valid for 2 years.

Precautions

1. The agarose should be stored in the supplied storage solution and protected from drying.
2. Before removing Protein G agarose from the storage container, thoroughly resuspend the slurry to ensure a homogeneous suspension. Avoid generating air bubbles during handling.
3. This product is intended for scientific research use only by qualified professionals. It must not be used for clinical diagnosis or treatment, food or pharmaceutical applications, and must not be stored in residential environments.
4. For your safety and health, please wear a lab coat and disposable gloves during operation.