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Protein A/G Agarose

1 Packing list

Components	HY-K0230-5 mL	HY-K0230-10 mL	HY-K0230-50 mL
Protein A/G Agarose	5 mL	10 mL	10 mL × 5

2 Introduction

MCE Protein A/G Agarose is an affinity chromatography medium for separation and purification of immunoglobulins.
MCE Protein A/G Agarose consists of purified Protein A/G recombinant fusion protein that has been covalently immobilized onto high-quality cross-linked 4% beaded agarose, binds nearly all isotypes and mammalian species of IgG from serum, ascites fluid, cell culture supernatant and other antibody samples.
The product contains 0.5 mL of gel per 1 mL of total volume. Prior to use, ensure the gel is thoroughly resuspended and mixed before aspiration.

Features of Protein A/G Agarose

- Protein A/G: immobilized recombinant fusion protein of the antibody-binding domains of Protein A and Protein G enables polyclonal IgG purification from nearly any mammalian species
- Inert and stable: superior manufacturing method immobilizes Protein A/G by charge-free, leach-resistant covalent bonds, resulting in low nonspecific binding and enabling multiple uses without decline in yield

3 Characteristics

Composition	4% cross-linked agarose
Ligand	Protein A/G
Binding Capacity	10-15 mg Rabbit IgG/mL of Resin
Bead Diameter	45-165 μm
Pressure	0.3 MPa, 3 bar
Storage Solution	1× PBS containing 20% ethanol

4 General Protocol

Buffer Preparation

Binding/Washing Buffer	0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0
Elution Buffer	0.1 M Glycine, pH 3.0
Neutralization buffer	1 M Tris-HCl, pH 8.0
Storage Buffer	1× PBS, 20% ethanol

Note: It is recommended to prepare all buffers with ultrapure water and. After preparation, filter them through a 0.45 μm or 0.22 μm membrane for sterilization.

Protein purfication

It is recommended to filter the sample with a 0.22 μm or 0.45 μm filter before purification.

Medium-pressure chromatography

1. Load the column: Load Protein A/G Agarose in an appropriate chromatography column and connect it to the chromatography system.
2. Equilibration: Equilibrate the column by washing it with 5× column volume of Binding Buffer. Repeat 2-3 times.
3. Loading: Loading sample using a pump or sample loops, collecting the effluent. Repeating this loading process can improve the binding efficiency.
   Note: a. Please choose a suitable gel volume according to the amount of protein to avoid exceeding the loading capacity of agarose.
   b. An increase in sample viscosity or volume may lead to column backpressure.
4. Washing: Wash the column with approximately 15-30× column volume of Washing Buffer to remove nonspecifically adsorbed hetero-proteins and collect the wash effluent until the absorbance at 280 nm stabilizes.
5. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, collect the eluent and immediately neutralize its pH with Neutralization Buffer (1/10 volume of total eluent volume), the collected eluates contain the target protein.
   Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
6. Regeneration: Thoroughly elute the column with 5-10× column volume of Elution Buffer. Equilibrate the column with Washing Buffer.
   Note: The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.
7. Storage: Equilibrate the resin with 5-10× column volume of Storage Buffer, disconnected the column and store at 2-8°C.

Gravity column method

1. Load the column: Choose an appropriate volume of Protein A/G Agarose suspension according to the amount of samples and load it into the gravity chromatography column, eliminating the protective solution.
2. Equilibration: Equilibrate the column: Equilibrate the resin with 5× column volume of Binding Buffer, repeat 2-3 times.
3. Loading: Loading sample and collect the effluent. It is recommended that the samples maintain contact with the agarose at least 2 min.
   Note: Repeated loading can improve the binding efficiency.
4. Washing: Wash resin with 10-15× column volume of Washing Buffer to eliminate non-specifically adsorbed hetero-proteins, collect the effluent.
5. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, collect the eluent and immediately neutralize its pH with Neutralization Buffer (1/10 volume of total eluent volume), the collected eluates contain the target protein.
   Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
6. Regeneration: Thoroughly elute the column with 5-10× column volume of Elution Buffer. Equilibrate the column with Washing Buffer.
   Note: The agarose should be balanced with Washing Buffer immediately after acidic elution and the agarose should not be kept in the Elution Buffer for more than 20 min.
7. Storage: Wash the column with 5× the volume of Storage Buffer and store at 2-8°C.

Centrifugation

1. Preparation of Agarose: Choose an appropriate volume of Protein A/G Agarose suspension according to the amount of samples and transfer it into a tube, centrifuge at 1,000 rpm for 1 min and discard. Wash the resin with 5× the volume of Washing Buffer, centrifuge at 1,000 rpm for 1 min and discard the supernatant. Repeat 2-3 times.
2. Binding: Add the sample and incubate at 4°C for 2-4 h (or 37°C for 0.5-2 h).
3. Washing: Centrifuge at 1,000 rpm for 1 min and discard the supernatant (e.g., if required, save supernatant for subsequent analysis). Wash the resin with 5× the volume of Washing Buffer, centrifuge at 1,000 rpm for 1 min and discard the supernatant. Repeat 3-5 times.
4. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, incubate at room temperature for 5-10 minutes, centrifuge at 1,000 rpm for 1 min. The collected supernatant contains the target protein. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis. Repeat 2-3 times and collect the supernatant each time.
   Note: a. The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.
   b. The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
5. Regeneration and storage: Wash the resin with 5-10× the volume of Washing Buffer, then wash the resin with 5-10× the volume of ddH_2O. Finally, wash the resin with 2× the volume of Storage Buffer and store at 2-8°C.

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1. Preparation of Agarose
   1) Add the required amount of Protein A/G Agarose suspension to a 2 mL tube, centrifuge at 1, 000 rpm for 1 min and discard the supernatant.
   2) Add 500 μL of Binding Buffer and mix well, centrifuge at 1, 000 rpm for 1 min and discard the supernatant. Repeat 3-4 times.
2. Antibody Binding
   1) Introduce an appropriate volume of diluted antibody into the agarose, gently rotate the tube for 30 min at room temperature or 2 h at 4°C. Centrifuge at 1,000 rpm for 1 min and retain the supernatant for future analysis.
   2) Add 500 μL Washing Buffer to the agarose and mix well, rotate the tube for 3-5 min at room temperature eliminates hetero-proteins that have been non-specifically adsorbed. Centrifuge at 1,000 rpm for 1 min and discard the supernatant, and repeat 2-3 times.
3. Immunoprecipitation of Target Antigen
   1) Add the antigen-containing sample to the agarose and mix well, then rotate the tube for 30 min at room temperature or leave overnight at 4°C.
      Note: a. If the binding between the antigen and the antibody is weak, prolong the incubation period accordingly.
      b. It is recommended to add protease inhibitors to prevent protein degradation.
   2) Centrifuge at 1,000 rpm for 1 min and collect the supernatant for future analysis.
4. Washing
   1) Add 1 mL Washing Buffer to the agarose and mix thoroughly, centrifuge at 1,000 rpm for 1 min and discard the supernatant. Repeat 2-3 times until the OD_{280} of the supernatant liquid is < 0.05.
   Note: If the OD_{280} of the supernatant liquid is > 0.05, increase the number of washes.
   2) Add 1 mL Washing Buffer to the agarose again, mix well and transfer to a new tube. Centrifuge at 1,000 rpm for 1 min and discard the supernatant.
5. Elution
   Two recommended elution methods depend on protein characteristics or further usage.
   1) Acidic condition Elution with Elution Buffer: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.
      Add 100-200 μL of Elution Buffer to the tube. Mix well and incubate for 10 min at room temperature, centrifuge at 1,000 rpm for 1 min and transfer the supernatant to a new tube. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis.
   Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
   2) Gel Electrophoresis and Immunoblotting Elution with SDS-PAGE Loading Buffer.
      Add 20-50 μL of 2× SDS-PAGE Loading Buffer to the tube. Mix well and heat for 5 min at 95ºC. Centrifuge at 1,000 rpm for 1 min. Reserve the supernatant for SDS-PAGE analysis.
      Note: As regular SDS-PAGE Loading Buffer contains β-mercaptoethanol and DTT, which can break the light and heavy chains of the antibody in the packing. And the SDS-containing Loading Buffer can denature the medium ligand. Anti-GFP Affinity Gel cannot be reused after denaturing elution.

5 Storage

4ºC, 2 years

Do not freeze

6 Precautions

1. Please resuspend the gel thoroughly before use.
2. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No. HY-K0010, HY-K0011) are highly recommended.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.