Antagene Inc.
Your Source of Antibody

Product Information Sheet

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Protein A Beads

Catalog # B0001-5
Size 5ml, 10ml, 25ml

Introduction

Protein A binds to most human and mouse IgG subclasses (e.g. human IgG1, IgG2, IgG3, IgG4, IgA; mouse IgG1, IgG2a, IgG2b, IgG3). It also binds to rat IgG1, IgG2c; goat IgG1, IgG2; sheep IgG1, IgG2; cow IgG1, IgG2; horse IgG (ab), IgG(c). Protein A binds strongly to total IgG from rabbit, dog, cat, pig, guinea pig.

This product can be used for 100-200 times. For frequent use, an aliquot can be stored at 4ºC for 1 month with addition of 0.02% sodium azide (NaN_3) to the storage buffer. Because this product can purify IgG subclasses from several species of mammals (see above), customers can conjugate the purification products they got with Sepharose™ 4B beads to purify secondary antibody.

Note: 20% ethanol was contained as protection solution in this product, please wipe off the ethanol before use.

Protein A Beads Specifications

Matrix: CNBr-activated Sepharose™ 4FF
Beads concentration: 1-2 mg/ml
Coupling conditions of matrix: pH 7-9, 4°C to 25°C, 2-16 h
Binding capacity: 4-7 mg IgG per ml
Bead size range: 45–165 µm
Mean bead size: 90 µm
Bead structure: Highly cross-linked agarose, 4%
Max. flow rate: 4 ml/min/cm^2
Recommended flow rate: 1-3 ml/min/cm^2

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Stability of the matrix: pH 3-11 (ligand dependent)

Storage: Store at 4℃ for frequent use, at -20℃ for at least one year.

Protocol

A: Buffers preparation

- Equilibration buffer A: 1% Nacl+0.1% Na_2HPO_4, pH≈7.5
- Equilibration buffer B: 1% CH3COONa adjusted pH to 5 by CH_3COOH.
- Elution buffer: 2% table sugar adjusted pH to 2-3 by CH_3COOH.
- Wash buffer: purified water
- Storage buffer: 30% glycerol

B. Sample preparation

1. Dilute the serum with equilibration buffer A to ensure its content and pH closed to equilibration buffer A.
2. Centrifuge diluted serum supernatants to sediment debris.
3. Filter supernatants through 0.45μm filter.

C. Affinity-purification

1. Load the Protein A beads into the empty column.
2. Wash column with Wash buffer in 3-5 column volumes to remove the glycerol, and then, equilibrate column by washing with Equilibration buffer A in 5-10 column volumes.
3. Bring the sample to room temperature, and load it into the column by a syringe or a pump. The total volume of the sample applied is not critical in most cases.
4. Load the sample into the column and collect the flow liquid, repeat this action for 3-5 times. If necessary, repeat for more times, then deal with the collected liquid reasonably.
5. Wash the column with Equilibration buffer B to remove other proteins.
6. Elute with Elution buffer, collect the flow liquid (antibody), adjust its pH by saturated Na_2CO_3 during collection. Then, customers can test the related data of the antibody as their own requirements.

D. Re-equilibration and Storage

1. Add 5-10ml Elution buffer to column to elute thoroughly, then neutralizate the column with Equilibration buffer A.
2. Wash the column bed with Storage buffer in 3-5 column volumes, seal the bottom of the column and store at -20℃ for at least one year. For frequent use, an aliquot can be stored at 4ºC for 1 month with addition of 0.02% sodium azide (NaN_3) to the storage buffer.