Protein G Beads

Catalog # B0002-5

Size 5ml

Introduction

Protein G binds to most IgG subclasses from human (human IgG1, IgG2, IgG3, IgG4), mouse (mouse IgG1, IgG2a, IgG2b, IgG3) and rat (rat IgG1, IgG2a, IgG2b, IgG2c) species. It can binds strongly to goat (total Ig, IgG1, IgG2), sheep (total Ig, IgG1, IgG2), cow (total Ig, IgG1, IgG2). Protein G can bind slightly with total Ig from rabbit, dog, cat, pig and 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.

Protein G 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
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 G 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 4°C for 1 month with addition of 0.02% sodium azide (NaN_3) to the storage buffer.