

Product Information

Protein A Sefinose

Product information for BSP096-25/ BSP096-5/ BSP096-100

Introductions

Protein A Sefinose is useful for affinity purification and isolation of IgG. Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgGs mainly through Fc regions. The recombinant protein A contains mainly high affinity ($K_a = 10^8/M$) IgG binding domains while other non-essential domains are removed to reduce nonspecific binding. Protein A Sefinose has been used to isolate and purify classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media.

Features

1. High IgG-binding capacity
2. Milder elution condition than protein G resin
3. Resin is reusable up to 10 times with no significant loss of binding capacity.

Characteristics

| | |
|----------------------------------|-----------------------------------------------------------------|
| Ligand | Recombinant Staphylococcal protein A produced in <i>E. coli</i> |
| The Numbers of IgG binding sites | 5 per ligand |
| MW of ligand | Approximately 43 kDa |
| PI of ligand | 5.17 |
| Degree of substitution | Approximately 2 mg protein A/ml |
| Static binding capacity | >20 mg Porcine IgG/ml drained medium |
| Stability | 2-8°C, six months |
| Matrix spherical | Agarose, 4% cross-linked |
| Average particle size | 90 μ m (45-165 μ m) |
| Storage solution | 1X PBS containing 20% ethanol |
| Storage conditions | 2-8 °C |

Immunoglobulin purification procedures

Before use, prepare the following two solutions:

1. Binding/wash buffer: NaCl 0.15 M, Na₂HPO₄ 20 mM, pH 7.4
 2. Elution buffer: Citric acid 0.1 M, pH 2.5-3.0
- Or use the BBI products: Binding/wash buffer(BSP048-2), Elution buffer(BSP048-1)

This procedure is optimized for a column of 0.5 ml bed volume. The volumes of the reagents can be scaled up or down according to the size of the column.

1. Sample Preparation: To ensure that proper ionic strength and pH are maintained for optimal

binding, it is necessary to dilute serum samples, ascite fluid or tissue culture supernatant at least 1:1 with binding/wash buffer. Alternatively, the sample may be dialyzed overnight in binding/wash buffer.

2. Column and Resin Preparation: Mix the slurry by gently inverting the bottle several times to suspend the resin completely. Use a pipette to transfer 1 ml of Protein A Sefinose slurry to a column, in which 1 ml of binding/wash buffer was added in advance. Allow the resin to settle and the storage buffer to drain from the column followed by the equilibration with 5 ml of binding/wash buffer.
3. Sample Purification: Gently apply the sample onto the resin with a flow speed of about 1 ml/min. Wash the column with 30 ml of binding/wash buffer, or until the absorbance of elute at 280 nm is stable.
4. Elute the antibody with 10-15 ml of elution buffer. Immediately neutralize the eluted fractions with 1 M Tris-HCl, pH 8.5 to pH 7.4.
5. Regeneration of the Column: Regenerate column by washing it with 10 ml of elution buffer followed by equilibration of the column with 5 ml of binding/wash buffer. Columns can be regenerated up to 10 times without significant loss of binding capacity.
6. Resin Storage: Store Protein A Sefinose in binding/wash buffer containing 20% ethanol at 2°C to 8°C. Do not freeze.

Immunoprecipitation(IP) procedure

1. To prepare Protein A Sefinose, wash the beads twice with PBS and restore to 50% slurry with PBS. It is recommended that the pipette tip be cut off when manipulating agarose beads to avoid disruption of the beads.
2. Pre-clear the cell lysate by adding 100 µl of protein A slurry (50%) per 1 ml of cell lysate and incubating at 4°C for 10 minutes on a rocker or orbital shaker. Pre-clearing the lysate will reduce non-specific binding of proteins to the resin when it is used for further assay.
3. Remove the Protein A Resin by centrifugation at 12,000 g at 4°C for 10 minutes. Transfer the supernatant to a fresh centrifuge tube.
4. Determine the protein concentration of the cell lysate (to perform a Bradford assay, one must dilute the cell Lysate at least 1:10 before determining the protein concentration because of the interference of the detergents in the lysis buffer with the Coomassie-based reagent).
5. Dilute the cell lysate to approximately 1 µg/µl total cell protein with PBS to reduce the concentration of the detergents in the buffer. To immunoprecipitated proteins that tend to express at low concentrations, use a more concentrated cell lysate (perhaps 10 µg/µl).
6. Add the recommended volume of the immunoprecipitating antibody to 500 µl (500 µg) of cell lysate. The optimal amount of antibody for quantitative immunoprecipitation of the protein of interest must be empirically determined for each sample.
7. Gently rock the cell lysate/antibody mixture for either two hours or overnight at 4°C on either a rocker or an orbital shaker. A two-hour incubation time is recommended for the immunoprecipitation of active enzymes in kinase and phosphatase assays.
8. Capture the immunocomplex by adding 100 µl protein A slurry (50 µl packed beads) and gently rocking on either a rocker or orbital shaker for either 1 hour or overnight at 4°C.
9. Collect the resin by centrifugation at 12,000 rpm. Discard the supernatant and wash the beads three times with 800 µl ice-cold RIPA or PBS buffer.

10. Resuspend the resin in 60 μ l 2X sample buffer and mix gently. This will allow for sufficient volume to run three lanes.
11. Boil the resin for five minutes to dissociate the immunocomplexes from the beads. The beads are removed by centrifugation and SDS-PAGE is performed with the supernatant.

Troubleshootings

| Problems | Possible Reasons | Suggestions |
|-----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Flow of the column is exceedingly slow (i.e., <0.5 ml/minute) | Gas present in buffers or sample on the column, which results in blockage of gel pores with microscopic air bubbles | Degas buffers and remove air bubbles from column |
| Antibody purified, but no specific antibody of interest detected | Antibody of interest is at very low concentration | Use serum-free medium for cell supernatant samples Affinity purify the antibody using the specific antigen coupled to an affinity support. |
| Antibody of interest purified, but it is degraded (as determined by lack of function in downstream assay) | Antibody is sensitive to low-pH Elution Buffer | Increase pH of Elution Buffer. |
| | Downstream application is sensitive to neutralized Elution Buffer | Desalt or dialyze eluted sample into suitable buffer |
| No antibody detected in any elution fraction | Sample devoid of antibody species or subclass that binds to Protein A | Refer to the Binding Characteristics Table for Protein A |