Protein A Sefinose™

Product information for BSP095-1/ BSP095-3/ BSP095-7/ BSP095-8/BSP048：

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 five high affinity (Ka = 10^8/M) IgG binding domains with other non-essential domains removed to reduce nonspecific binding.Protein .Protein A sefinose™ has been used to isolate and purify classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. Since only the Fc region is involved in binding, the Fab region is available for binding antigens. These qualities of protein A sefinose™ combined with BBI precision and affordability make , and can be used for the isolation of protein complexes by immunoprecipitation.

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 E. coli
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 μm (45-165 μm)
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 insure 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 against 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 immunopecipitation 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 µl 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)	Outgassing of 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
Considerableantibody purified, but no specific antibody of interest	Antibody of interest is at very low concentration	Use serum-free medium for cell supernatant samples
		Affinity purify the antibody using

detected		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