Product Information

Protein A-Agarose

Lyophilized powder

P1406

Product Description

Protein A is a highly stable cell surface receptor that occurs in several strains of *Staphylococcus aureus*. Protein A consists of a single polypeptide chain with a molecular weight of ~42 kDa, with four repetitive domains rich in Asp and Glu, but devoid of Cys. Protein A contains little or no carbohydrate, only 4 Tyr residues, and no Trp.[1-3]

Protein A can bind to the Fc portion of immunoglobulins, especially IgGs, from many species.[3] This aspect of Protein A makes it useful for IgG purification,[4-10] especially when conjugated to an inert solid support such as agarose. Protein A has an optimal binding capacity in the range of pH 8-9.

This product contains Protein A that has been coupled to cyanogen bromide-activated 4% cross-linked agarose. Its binding capacity is in the range of 20-30 mg/mL of human IgG. Several publications,[11-13] theses,[14] and dissertations[15] have cited use of P1406 in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Buffers

Buffer A:
0.02 M NaH_2PO_4 (such as Cat. No. S3139): 2.4 g
0.15 M NaCl (such as Cat. No. S3014): 8.8 g
Adjust volume to 1 liter with water. Adjust pH to 8.0.

Buffer B:
0.2 M Na_2HPO_4 (such as Cat. No. S3264): 25.7 mL
0.1 M Citric Acid (such as Cat. No. C7129): 24.3 mL
Water: 50.0 mL
The pH depends on the species/subclass. See Table 1.

Swelling the Resin

- Lyophilized products should be swollen in Buffer A for 30 minutes or longer at room temperature.
- **Do not stir with any kind of mechanical stirrer.**
- One gram of powder typically swells to 3-4 mL of hydrated gel.
- Resins can be reused at least 5 times if stored and handled properly.

Storage/Stability

Store lyophilized powders at –20 °C. Store suspensions and hydrated resins at 2-8 °C in Buffer A with either 0.1% sodium azide, 20% ethanol, or 1% toluene as preservative. **Do not freeze liquid suspensions of Protein A-Agarose.**

Procedure

Please refer to Reference 4 for a review of Protein A binding to immunoglobulins (including extensive tables).[4] Reference 4 also covers immunoglobulin levels in sera.

Note: Tyrosine residues in the Fc region of IgG are involved with Protein A interactions. Glycyltyrosine may be used for elution (0.1 M glycyltyrosine in 2% NaCl, pH 7.0, at room temperature).[16]

Column Method

If the solution volume is significantly greater than the resin volume, the Column Method is recommended.

- Make a 1:1 suspension of resin in Buffer A.
- Pour into column.
- Allow column to flow as it is settling.
- After the column has settled, wash with 20 column volumes (CV) of Buffer A.
- Apply sample.
- Wash with 10 CV of Buffer A.
- Elute with 3 CV of Buffer B. Collect fractions.
- Neutralize the eluate with 0.1 M NaOH.
- Assay the eluate for IgG.
- Re-equilibrate the column with 20-30 CV of Buffer A.
- Store in Buffer A with a preservative at 2-8 °C.

Batch Method

- Equilibrate resin on a sintered glass funnel or Buchner funnel (with Whatman® 54 filter paper) by washing with 10 resin volumes (RV) of Buffer A using gentle vacuum.
- Combine resin and sample solution in a container.
- Gently mix suspension on a shaker for 1 hour, or for longer if the solution volume is significantly greater than the resin volume.
- Collect the resin on the sintered flask or Buchner funnel. Wash with 10 RV of Buffer A.
- Transfer the resin to a beaker. Add twice the RV of Buffer B.
- Gently mix on shaker for 15 minutes.
- Collect resin on funnel as before, using a clean sidearm flask to collect the eluted antibody.
- Bring the eluate to neutral pH with 0.1 M NaOH.
- Wash the resin with 20 RV of Buffer A.
- Add preservative and store at 2-8 °C.

Cleaning Procedure

A decrease in the binding capacity may be due to steric hindrance by non-specifically bound proteins. It may be possible to clean the resin by washing the resin with:

- 10-20 volumes of 100 mM Tris or borate buffer, pH 8.5, containing 0.5-2.0 M NaCl,
- followed by 10-20 volumes of 100 mM acetate buffer, pH 4.0, containing 0.5-2.0 M NaCl.

Re-equilibrate the resin with 20 volumes of Buffer A. Add preservative and store at 2-8 °C.

References

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Table 1. Immunoglobulin Binding

Species	Subclass	Binding Capacity	Elution pH
Human	IgG	High	4
	IgG1	High	3.9 - 4.6
	IgG2	High	4.3 - 5
	IgG3	----	
	IgG4	High	3.9 - 5
Mouse	IgG1	Low (*)	6 - 7
	IgG2a	High	4.5 - 5
	IgG2b	High	4.5
	IgG3	High	3.5 - 4
Rabbit	IgG	High	3
Rat	IgG1	Low (*)	7
	IgG2a	----	
	IgG2b	----	
	IgG2c	Medium-High	3 - 4
Guinea Pig	IgG	High	4
Bovine	IgG	Low	
Goat	IgG	---- (*)	

(*) Capacity may be increased by using alternative buffers, such as:

- 1 M glycine with 2 M NaCl, pH 9, or
- 1 M borate with 2 M NaCl, pH 9.

With mouse IgG1:

- Use a higher pH (such as pH 9), and a sodium chloride concentration of 2-3 M.
- Elute with a gradient to pH 3 and to 0.15 M NaCl.

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