

Product Information

Protein A-Agarose macrobeads

Aqueous suspension

P1925

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 is an aqueous suspension of Protein A-Agarose in 0.9% NaCl, with 15 mM sodium azide as a preservative. The Protein A has been coupled to cyanogen bromide-activated 6% Sepharose® macrobeads.^{11,12} Its binding capacity is in the range of ~6 mg/mL of human IgG. Several publications have cited use of P1925 in their protocols.¹¹⁻¹⁴

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 NaH₂PO₄ (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 Na₂HPO₄ (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.

Do not stir with any kind of mechanical stirrer. Resins can be reused at least 5 times if stored and handled properly.

Storage/Stability

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).¹⁵

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

1. Björk, I. *et al.*, *Eur. J. Biochem.*, **29(3)**, 579-584 (1972).
2. Goding, J.W., *J. Immunol. Methods*, **20**, 241-253 (1978).
3. Boyle, M.D.P. and Reis, K.J., *Bio/Technology*, **5**, 697-703 (1987).
4. Lindmark, R. *et al.*, *J. Immunol. Methods*, **62(1)**, 1-13 (1983).
5. Langone, J.J., *J. Immunol. Methods*, **51(1)**, 3-22 (1982).
6. Ey, P.L. *et al.*, *Immunochem.*, **15(7)**, 429-436 (1978).
7. Surolia, A. *et al.*, *Trends Biochem. Sci.*, **7(2)**, 74-76 (1982).
8. Ishikawa, E., and Kato, K., *Scand. J. Immunol.*, **8(s7)**, 43-55 (1978).

9. Werner, S., and Machleidt, W., *Eur. J. Biochem.*, **90(1)**, 99-105 (1978).
10. Tucker, D.F. *et al.*, *J. Immunol.*, **121(5)**, 1644-1651 (1978).
11. Cullin, C., and Minvielle-Sebastia, L., *Yeast*, **10(1)**, 105-112 (1994).
12. Wagner-Hülsmann, C. *et al.*, *Glycobiology*, **6(8)**, 785-793 (1996).
13. Liu, N. *et al.*, *J. Nutr. Biochem.*, **18(9)**, 597-608 (2007).
14. Hlavová, M. *et al.*, *PLoS One*, **6(5)**, e19626 (2011).
15. Bywater, R., in *Chromatography of Synthetic and Biological Polymers* (Epton, R., ed.). Ellis Horwood (Chichester, UK), pp. 337-340 (1978).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://www.sigmaaldrich.com/techservice).

Standard Warranty

The applicable warranty for the products listed in this publication may be found at [SigmaAldrich.com/terms](https://www.sigmaaldrich.com/terms).

Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://www.sigmaaldrich.com/offices).



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.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, Millipore, MILLIPLEX and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

P1925pis Rev 01/22 CMH,TW,SBC,MAM,GCY

