Product Information

Protein A-Sepharose® 4, Fast Flow from *Staphylococcus aureus*

Aqueous ethanol suspension

P9424

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 Sepharose® 4B Fast Flow (a cross-linked, 4% beaded agarose). Its binding capacity is ≥30 mg/mL of human IgG. Several publications,[11-21] theses[22,23] and dissertations[24-47] have cited use of P9424 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.

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).[48]

Column Method

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

- 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. Ogrin, C. *et al*., *Endocr. Pract.*, **21(8)**, 966-971 (2015).
12. Lochhead, P.A. *et al.*, *Cell Cycle*, **15(4)**, 506-518 (2016).
13. Oliveiro, G. *et al*., *Mol. Cell. Proteomics*, **15(11)**, 3450-3460 (2016).
14. Rao, K.H. *et al*., *mSphere*, **1(4)**, e00080-16 (2016).
15. Baptista, T. *et al*., *Mol. Cell*, **68(1)**, 130-143.e5 (2017).
16. Saint-Germain, E. *et al.*, *Aging (Albany NY)*, **9(10)**, 2137-2162 (2017).
17. Alsafani, M. *et al*., *Mol. Cell Biol.*, **38(23)**, e00222-18 (2018).
18. Burslem, G.M. *et al*., *Cell Chem. Biol*., **25(1)**, 67-77.e3 (2018).
19. Shapira, K.E. *et al*., *Mol. Biol. Cell*, **29(20)**, 2494-2507 (2018).
20. Alsulami, M. *et al*., *Mol. Cell Proteomics*, **18(7)**, 1428-1436 (2019).
21. Jiang, J. *et al.*, *Methods Mol. Biol.*, **2372**, 19-26 (2021).
22. Tømmerås, Berit, "Analysis of glomerular target structures for nephritic anti-dsDNA antibodies playing central roles in lupus nephritis". University of Tromsø, M.S. thesis, p. 87 (2008).
23. Becker, Abigail Harris, "Epigenetic and Gene Expression Changes Mediated by Histone H3 Methylation in Acute Myeloid Leukemia". The Pennsylvania State University, M.S. thesis, pp. 28, 64 (2018).
24. Zhu, Cong, "Gene Regulatory Networks of Agl15, A Plant MADS Transcription Factor". University of Kentucky, Ph.D. dissertation, p. 54 (2005).
25. Pastushok, Landon, "Characterization of the Ubc13-Mms2 Lysine-63-Linked Ubiquitin Conjugating Complex". University of Saskatchewan, Ph.D. dissertation, p. 58 (2006).
26. Ragan, Emily J., "Immune-Related Protein Complexes and Serpin-1 Isoforms in *Manduca Sexta* Plasma". Kansas State University, Ph.D. dissertation, p. 118 (2008).
27. Ciglar, Lucia, "*Drosophila* myogenesis as a model for studying cis-regulatory networks: identifying novel players and dissecting the role of transcriptional repression". Ruperto-Carola Universität Heidelberg, Dr. rer. nat. dissertation, p. 66 (2010).
28. Cunha, Paulo Miguel Fernandes, "Combinatorial Regulation of Shared Target Genes by *Lmd* And *Mef2*". Phillips-Universität Marburg, Dr. rer. nat. dissertation, p. 50 (2010).
29. Jeevan-Raj, Beena Patricia, "Molecular mechanisms underlying AID target specificity during antibody diversification". Université de Strasbourg, Ph.D. dissertation, Appendix I, p. xxxii (2011).
30. Wong, Jia Lih, "Identification of Deubiquitylases Involved in the Regulation of Adherens Junction Components". University of Liverpool, Ph.D. dissertation, p. 84 (2012).
31. Mohan, Bindu, "Role of Siglec-7 in ganglioside recognition and modulating NK cell biology". University of Dundee, Ph.D. dissertation, p. 41 (2013).

32. Müller, Martin, "Characterization of the MYST histone acetyltransferase MYS-2 in *Caenorhabditis elegans*". Universität Duisburg-Essen, Dr. rer. nat. dissertation, p. 48 (2013).
33. Chan, Waipan, "Molecular Mechanisms of Mutant CARD11 Signaling to Nf-κb in Human Diffuse Large B Cell Lymphoma". Johns Hopkins University, Ph.D. dissertation, pp. 18, 65 (2014).
34. McCartney, Amber Joyce, "The Dynamic Neuron: Cellular Mechanisms Maintaining Neural Activity and the Consequences of Phosphatidylinositol 3,5-bisphosphate Biosynthesis on Synapse Function". University of Michigan, Ph.D. dissertation, p. 70 (2014).
35. Bagger, Frederik Otzen, "Aberrant Gene Expression in Acute Myeloid Leukaemia". University of Copenhagen, Ph.D. dissertation, pp. 202, 219 (2014).
36. Averkov, Volodymyr, "Tissue-specific roles of Snail-like transcription factors in the embryogenesis of *Drosophila melanogaster*". Universität zu Köln, Ph.D. dissertation, p. 40 (2015).
37. Gauson, Elaine J., "Regulation of viral and host genomes by high risk human papillomavirus E2 protein in association with cellular factors". University of Glasgow, Ph.D. dissertation, p. 88 (2015).
38. McQuay, Amy Brook, "The Role of Sialic Acid Acetylesterase in the Maintenance of B Cell Self Tolerance". Harvard University, Ph.D. dissertation, p. 61 (2015).
39. Huang, Yu-Hung, "The Role of HuR in Mediating Ovarian Cancer Treatment". Drexel University College of Medicine, Ph.D. dissertation, p. 111 (2016).
40. Concilli, Mafalda, "HSP70 as a Target for Correction of Wilson Disease Causing ATP7B Mutants". The Open University, Ph.D. dissertation, p. 31 (2017).
41. Li, Huihui, "Role of the p53/miR-34a Pathway in the Response to Tumor Hypoxia". Ludwig-Maximilians-Universität München, Dr. rer. nat. dissertation, p. 41 (2017).
42. Smolenski, Grant Alan, "The bovine teat canal: Its role in pathogen recognition and defence of the mammary gland". University of Waikato, Ph.D. dissertation, p. 63 (2018).
43. Korzystka, Aleksandra, "*In vitro* functional studies of the tumour vascular target PCDH7 and identification of novel tumour vascular targets in colorectal cancer". University of Birmingham, Ph.D. dissertation, p. 63 (2018).
44. Zeng, Nina, "Adaptations of Amino Acid Transporters and Sestrins to Exercise, Nutrition and Ageing in Human Skeletal Muscle". University of Auckland, Ph.D. dissertation, p. 82 (2018).
45. Festa, Beatrice Paola, "Mechanistic Insights & Therapeutic Approaches in Endolysosomal Disorders Affecting the Kidney Proximal Tubule". Universität Zürich, Dr. sc. nat. dissertation, p. 91 (2019).
46. Oliveira, Marta Sofia dos Santos, "Regulation of alternative polyadenylation in *Drosophila melanogaster*". Universidade do Porto, Ph.D. dissertation, p. 43 (2019).
47. Rioja, Alba Lopez, "The role of srGAP2 in the regulation of endothelial function". University of Bristol, Ph.D. dissertation, p. 83 (2021).
48. Bywater, R., in *Chromatography of Synthetic and Biological Polymers* (Epton, R., ed.). Ellis Horwood (Chichester, UK), pp. 337-340 (1978).

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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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