Product Information

Protein A-Sepharose® from *Staphylococcus aureus*

Lyophilized powder

P3391

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® CL-4B. Its binding capacity is ~20 mg/mL of human IgG. Several publications,[11-17] theses[18-24] and dissertations[25-43] have cited use of P3391 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).[44]

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. Li, X. *et al*., *Plant Cell*, **10(1)**, 119-130 (1998).
12. Hennen-Bierwagen, T.A. *et al.*, *Plant Physiol.*, **146(4)**, 1892-1908 (2008).
13. Hayes, N.V.L. *et al*., *PLoS One*, **6(12)**, e28271 (2011).
14. Flici, H. *et al*., *Nat. Commun.*, **5**, 4484 (2014).
15. Cho, K.-J., and Roche, P.A., *Methods Mol. Biol.*, **1988**, 271-277 (2019).
16. Sanna, S. *et al.*, *Cell Death Dis.*, **11(5)**, 369 (2020).
17. Wright, W.W. *et al*., *J. Cell Signal.*, **2(1)**, 9-26 (2021).
18. Gines, Leoned G., "Analysis of the Bactericidal Activity of Antiserum Raised Against the N-terminal Half of an 85-kilodalton Outer Membrane Protein of *Neisseria meningitidis*". Montana State University, M.S. thesis, pp. 15, 16 (2002).
19. Shen, Fei, "Effects of E2 on ApoE Secretion and Neurite Outgrowth in Cultured Adult Mouse Cortical Neurons". Eastern Illinois University, M.S. thesis, p. 10 (2002).
20. O'Connoll, Marie, "Development of antibodies to human transient receptor potential vanilloid 1 for future targeting of therapeutics to sensory neurons". Dublin City University, M.Sc. thesis, p. 30 (January 2014).
21. Wilson, Robyn M., "Trimethylated Lysine 4 at Histone 3 Shows the Same Circadian Rhythm at Promoters of Diversely-Expressed Genes in *Chlamydomonas Reinhardtii*". Western Kentucky University, M.S. thesis, p. 10 (2016).
22. Jacob, Arthur, "Proteomic analysis of protein arginine methyltransferases 5 and 7 using BioID method". McGill University, M.Sc. thesis, p. 26 (2016).
23. Carswell, Matthew, "A Regulatory Role for 14-3-3 Proteins in the Starch Biosynthetic Pathway of *Zea mays*". University of Guelph, M.Sc. thesis, p. 95 (2021).
24. Constantinou, Jason, "A mutation in the semaphorin signalling pathway and its consequences in prostate cancer". University College London, MD(Res) thesis, p. 97 (2010).
25. MacLean, Christine Anne, "Herpes Simplex Virus DNA-Binding Proteins: Studies on 21K and the 'a' Sequence". University of Glasgow, Ph.D. dissertation, p. 86 (1987).
26. Mernaugh, Raymond LeRoy, "The production and characterization of anti-idiotypic antibodies to syngeneic monoclonal and xenogeneic polyclonal antibodies to soybean mosaic virus". Iowa State University, Ph.D. dissertation, p. 27 (1987).

27. Yang, Yi, "Signal transduction of abscisic acid in *Arabidopsis thaliana*: Identification and characterisation of protein interaction partners of ABI2". Technischen Universität München, Dr. rer. nat. dissertation, p. 43 (2003).
28. Kuo, Lillian S., "Structural and Functional Analysis of HIV-1 Nef Activation of Pak-2". University of Texas Southwestern Medical Center at Dallas, Ph.D. dissertation, pp. 45, 46 (2008).
29. Zettlitz, Kirsten Anja, "Engineered Antibodies for the Therapy of Cancer and Inflammatory Diseases". Universität Stuttgart, Dr. rer. nat. dissertation, p. 31 (2010).
30. Sollome, James Jerome, "Heregulin Activates a Novel HER2/HER3-MTK1-GIT1/ERK1/2 MAPK Signaling Pathway". University of Arizona, Ph.D. dissertation, pp. 56, 109 (2014).
31. Farrugia, Mark K., "The Diverse Relationship of Kruppel-Like Factors 4 and 5 in Breast Cancer". West Virginia University, Ph.D. dissertation, p. 101 (2015).
32. Homoud, Alyaa Munaji, "Characterisation of proteins in camel milk, the effect of heat treatment on physicochemical and functional properties related to yogurt". Heriot-Watt University, Ph.D. dissertation, p. 111 (June 2015).
33. Chang, Elizabeth Tsuying, "Heterogenous Ribonucleoprotein A18 (hnRNP A18) Promotes Tumor Growth by Increasing Protein Translation of Selected Transcripts in Cancer Cells". University of Maryland Baltimore, Ph.D. dissertation, p. 37 (2016).
34. Davidson, Adam S., "Investigation of vesicular stomatitis virus interaction with host cell proteins and associated mRNA structures". Wake Forest University, Ph.D. dissertation, p. 69 (May 2016).
35. Fegan, Jamie, "Evaluation of the Bacterial Transferrin Receptor as a Vaccine Antigen". University of Calgary, Ph.D. dissertation, p. 65 (2016).
36. Meier, Johannes Peter, "CD8+ T cell epitope-enriched HIV-1-Gag antigens with preserved structure and function". Universität Regensburg, Dr. rer. nat. dissertation, p. 66 (2016).
37. Mattiske, Tessa, "Investigating the Pathogenic Mechanism of Expanded Polyalanine Tract Mutations in the ARX Homeobox Transcription Factor causing Intellectual Disability". University of Adelaide, Ph.D. dissertation, p. 75 (2017).
38. Steinwand, Michael Allan, "Biochemical and Genetic Mechanisms Underlying Basal Salicylic Acid Synthesis In *Arabidopsis thaliana*". University of California Berkeley, Ph.D. dissertation, p. 84 (2017).
39. Barutcu, Seda, "Role of JIP1-JNK Signaling in Beta-Cell Function and Autophagy". University of Massachusetts Medical School, Ph.D. dissertation, p. 99 (2018).
40. Luff, Daisy, "Elucidating the molecular mechanisms of p110δ activation in T cell antigen receptor signalling". Cambridge University, Ph.D. dissertation, p. 145 (2018).
41. Alsina, Gemma Gou, "Molecular Mechanisms Underlying the Role of SynGAP in Cognition and Synaptopathies". Universitat Autònoma de Barcelona, Ph.D. dissertation, p. 103 (2019).
42. Llorens, Marc Herniaz, "Lipid raft association and subcellular localization of UNC5 Netrin-1 receptors". Universitat de Barcelona, Ph.D. dissertation, p. 137 (2019).
43. Nguyen, Thien Anh, "Sex-linked neuroligins and their roles at synapses". Georgetown University, Ph.D. dissertation, p. 39 (2020).
44. 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.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to 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.

P3391pis Rev 01/22 CMH,TW,SBC,MAM,GCY