Protein A AgaDex FF Affinity Resin

Product information

Product Description

Protein A AgaDex FF Affinity Resin is a fast-flow affinity resin for antibody purification. Recombinant Staphylococcus aureus Protein A, expressed in E. coli and free of animal-derived components, is coupled to 4% cross-linked agarose beads. The ligand retains all five IgG-binding domains of native Protein A (E, D, A, B and C), giving a dynamic binding capacity above 40 mg human IgG per mL of resin. The resin binds many antibody classes and subclasses from human, mouse, rat, rabbit, bovine and other species, and purifies antibodies and Fc-fusion proteins from ascites, serum, cell culture supernatants or cell lysates, from research to production scale.

Product Features

- High binding capacity: ≥40 mg human IgG/mL resin
- Fast flow: 60-300 cm/h recommended, up to 900 cm/h
- Recombinant, animal-origin-free Protein A ligand
- Easy to scale up from laboratory to production

Storage

Supplied in 20% ethanol. Store at 2-8 °C; do not freeze. Shipped at 2-30 °C. Shelf life: 3 years.

Safety Information

Always wear appropriate lab PPE: coat, gloves and goggles. Please consult the Material Safety Data Sheet (MSDS) for more information.

Note: This product is intended for research use only.

Technical Parameter

Parameter	Specification
Matrix	Cross-linked 4% agarose
Ligand	Recombinant Protein A (E. coli), ≥6 mg/mL resin
Particle Size Range	45-165 µm
Average Particle Size	~90 µm
Binding Capacity (DBC10%)	≥40 mg human IgG/mL resin (6 min residence time)
Recommended Flow Rate	60-300 cm/h
Maximum Flow Rate	900 cm/h at 0.3 MPa (10 cm bed height)
pH Stability	3-10 (working), 3-12 (short term)
Chemical Stability	Common aqueous buffers; 10-100 mM NaOH (CIP, ≤15 min contact); 6 M guanidine HCl; 70% ethanol
Storage Solution	20% ethanol
Storage Temperature	2-8 °C (shipped at 2-30 °C)

Note:

1. Binding capacity may vary with the sample source and antibody subclass.

2. Resin volumes refer to the settled resin volume.

Product Description	Catalog No.
Protein A AgaDex FF Affinity Resin (10 mL)	DWB01L-10
Protein A AgaDex FF Affinity Resin (20 mL)	DWB01L-20
Protein A AgaDex FF Affinity Resin (50 mL)	DWB01L-50
Protein A AgaDex FF Affinity Resin (100 mL)	DWB01L-100
Protein A AgaDex FF Affinity Resin (500 mL)	DWB01L-500
Protein A AgaDex FF Affinity Resin (1000 mL)	DWB01L-1000

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Protocol

A. Column Packing

The procedure below describes packing the resin in a column connected to a chromatography system.

1. *Temperature equilibration:* Bring all materials to the temperature at which the column will be run. Degas all liquids if possible.
2. *Resin volume:* Required settled resin volume = column volume (CV) × compression factor. The settled volume is the stable volume read after the resin has fully settled in its 20% ethanol storage solution. The recommended compression factor is 1.15; it can be reached either by lowering the column adapter (mechanical compression) or by packing at a high flow rate.
3. *Resin washing:* Mix the resin slurry thoroughly and measure out the required settled resin volume. Remove the storage solution by vacuum filtration, then wash the resin 3 times with about 3 bed volumes of purified water each time to remove the storage solution completely.
4. *Slurry preparation:* Transfer the resin to a suitable container and add packing buffer to make a 50-75% (v/v) slurry. Mix well before use.
5. *Column preparation:* Fill the bottom of a clean column with packing buffer to remove air from the bottom frit and outlet. Leave a small amount of liquid in the column, close the bottom outlet and mount the column vertically.
6. *Packing:* Pour the well-mixed slurry into the column in one continuous motion (use a packing reservoir if needed), letting it run down the inner wall of the column to avoid air bubbles. Fill the reservoir with packing buffer, close it and connect the column to the chromatography system.
7. *Compression:* Let the resin settle by gravity or at a low flow rate (50-150 cm/h). When the resin/liquid interface is clear, remove the packing reservoir, fit the top adapter and lower it to the interface. Continue packing at a high flow rate (see table below), keeping the column pressure below 0.3 MPa, until the bed height is stable, and mark this height. Stop the pump, open the top adapter valve and close the column outlet. Lower the adapter to the position given by the compression factor relative to the marked height and lock it. Packing is now complete; equilibrate the packed bed at a high flow rate before use.

Packing condition	Value
Compression factor	1.15
Packing flow rate	300-600 cm/h

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B. Column Efficiency Test

After packing and before use, confirm the packing quality by measuring column efficiency, expressed as the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As). Use acetone or NaCl as the tracer, with the conditions below:

Parameter	Acetone method	NaCl method
Sample	1.0% acetone	0.8-1.0 M NaCl
Sample volume	1.0% CV	1.0% CV
Mobile phase	Purified water	0.4 M NaCl
Flow rate	30 cm/h	30 cm/h
Detection	UV 280 nm	Conductivity

Calculate HETP, the number of theoretical plates (N) and As from the UV or conductivity trace:

$HETP = L/N \qquad N = 5.54 \times (VR/Wh)^2 \qquad As = b/a$

where L = bed height (cm); VR = volume eluted from the start of sample application to the peak maximum; Wh = peak width at half of the peak height; a and b = the front (ascending) and back (descending) parts of the peak width at 10% of the peak height. In general, HETP should be less than 3 times the mean particle size of the resin (HETP/D50 < 3) and As should be between 0.8 and 1.5.

C. Equilibration and Sample Loading

Phosphate buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0-7.4) is a common, versatile equilibration buffer, referred to below as Buffer A.

1. *Equilibration:* Before loading, equilibrate the column with 5-10 CV of Buffer A at the operating flow rate, until the conductivity and pH of the effluent are stable and match Buffer A.
2. *Loading amount:* Load about 50-80% of the DBC10% (mg of target protein per mL of resin). For human IgG (DBC10% about 40 mg/mL), the recommended load is 20-32 mg/mL. A lower load can be used during method development to check binding, specificity and elution.
3. *Sample preparation:* Before loading, centrifuge the sample (>10,000 × g) and filter it (0.22 or 0.45 µm) to prevent column clogging.
4. *Wash:* After loading, wash the column with 3-10 CV of Buffer A.

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D. Elution

1. *Elution buffer:* Glycine, citrate or acetate buffer at about pH 3 normally elutes antibodies completely.
2. *Optimizing pH:* Low pH can inactivate or aggregate some antibodies. During method development, lower the pH stepwise to find the highest pH that still gives acceptable recovery. Antibodies typically begin to elute at pH 4.5-6.
3. *Neutralization:* To shorten exposure to low pH, collect the eluted fractions into a weakly alkaline buffer for immediate neutralization.
4. *Final wash:* After elution, wash the column with 5-10 CV of elution buffer to make sure all bound antibody is removed before the next run or cleaning.

E. Cleaning-in-Place (CIP)

Clean the column if the back pressure increases, if strongly bound proteins cannot be eluted, or if precipitated or denatured proteins are present. Cleaning in the reverse flow direction is often more effective. Use one of the procedures below:

CIP procedure	Purpose
6 M guanidine hydrochloride, 2 CV; then Buffer A, 5 CV	Removes precipitates and denatured proteins
0.01-0.1 M NaOH, 10-15 min contact; then Buffer A, 5 CV	Removes precipitates and denatured proteins
0.1% non-ionic detergent, 2 CV; then Buffer A, 5 CV	Removes hydrophobically bound proteins
Or 70% ethanol, 3-5 CV; then Buffer A, 5 CV	Removes hydrophobically bound proteins

F. Storage

The resin is supplied in 20% ethanol and should be returned to 20% ethanol after use. Store at 2-8 °C. Do not freeze.

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Antibody Binding Profile

Protein A Binding Properties

The ligand of this resin retains the five IgG-binding domains (E, D, A, B and C) of native Staphylococcus aureus Protein A. Each domain consists of 58 amino acids folded into three anti-parallel α-helices. All domains bind the Fc region of IgG1, IgG2 and IgG4, but bind IgG3 only weakly. Binding involves non-covalent hydrophobic and polar interactions and hydrogen bonds under physiological conditions; the D domain can also bind the Fab region. Bound antibodies are usually eluted at low pH (about pH 3) with glycine, citrate or acetate buffer, which disrupts the interaction.

Recombinant Streptococcal Protein G also binds IgG. The table below compares the binding of Protein A and Protein G to antibody classes and subclasses from common species (– no binding; + weak; ++ moderate; +++ strong).

Species	Antibody class	Protein A	Protein G
Human	IgG1	+++	+++
	IgG2	+++	+++
	IgG3	+	+++
	IgG4	+++	+++
	IgM	+	–
	IgD	–	–
	IgA	+	–
	Fab	+	+
Mouse	IgG1	+	++
	IgG2a	+++	+++
	IgG2b	+++	+++
	IgG3	+++	+++
	IgM	–	–
Rat	IgG1	+	++
	IgG2a	–	+++
	IgG2b	–	+
	IgG2c	+++	+++
Cow	IgG1	+	+++
	IgG2	+++	+++
Goat	IgG1	+	+++
	IgG2	+++	+++
Horse	IgG(ab)	+	–
	IgG(c)	+	–
	IgG(T)	–	+++
Rabbit	IgG	+++	+++
Pig	IgG	+++	+
Dog	IgG	+++	+
Chicken	IgY	–	–

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Troubleshooting

Problem	Possible cause	Solution
Target protein does not bind, or binding capacity is low	Sample overload	Reduce the sample load
	Sample flow rate is too high	Lower the sample flow rate
	Aggregated proteins or lipids in the resin	Clean the resin thoroughly or replace it
	Incorrect pH of the sample or equilibration buffer	Check that the sample and buffer pH are within the recommended range
Low yield of target protein after elution	Target protein does not bind or binds weakly	Confirm that the target protein binds to Protein A
	Unsuitable elution conditions	Lower the elution buffer pH to 2.5-3.0
	Elution time is too short	Lower the flow rate to increase the elution residence time
	Elution volume is too low	Increase the elution volume
Target protein purity is low	Sample is too viscous	Dilute the sample with equilibration buffer
	Sample was not pretreated	Centrifuge and filter the sample before loading
	Protein or lipid impurities have precipitated in the resin	Clean the resin promptly (see CIP)
	Poor elution conditions, e.g. flow rate too high or gradient too steep	Optimize the elution conditions
	Target protein is degraded	Check the stability of the target protein
	Non-specific binding of impurities to the resin	Use suitable buffer additives to reduce non-specific binding
	Large dead volume above the resin bed	Repack the column or reduce the volume above the bed
	Microbial growth in the resin	Store the resin promptly in 20% ethanol after use
Binding capacity decreases over time	Sample flow rate is too high	Reduce the sample flow rate
	Proteins or lipids have accumulated in the resin	Clean the resin promptly
	Ligand oxidation or loss after extended use	Clean the resin or replace it with new resin
Fronting peak	Column is over-packed	Repack the column
Tailing peak	Column is under-packed	Repack the column
Low flow rate or high back pressure	Proteins or lipids have accumulated in the column	Clean the resin and column filters promptly
	Protein has precipitated in the resin	Adjust the equilibration and elution buffers to keep the target protein stable while maintaining binding
	Microbial growth in the column	Store the resin promptly in 20% ethanol after use

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