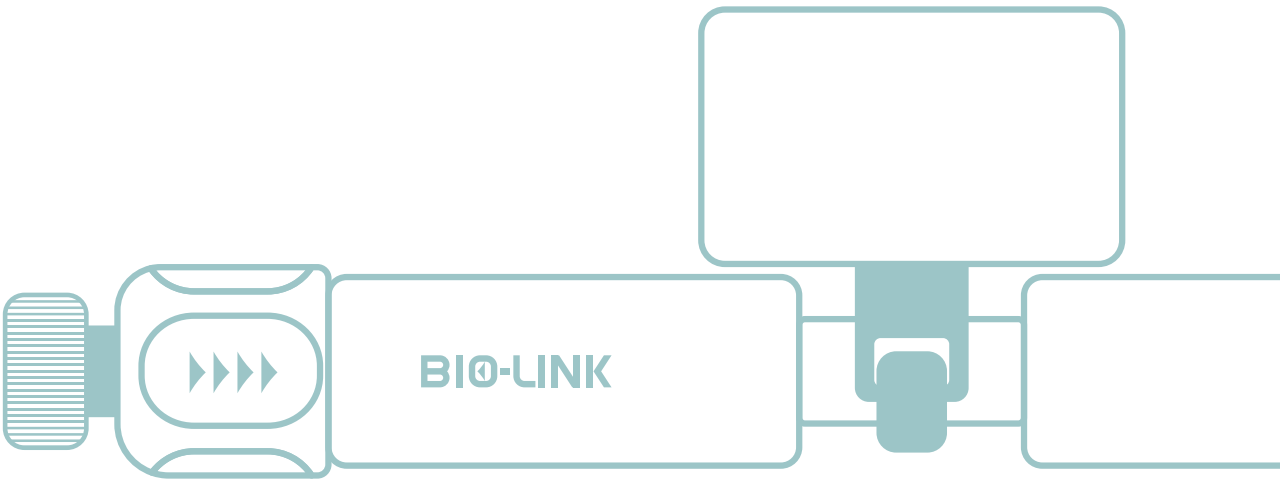
BIO-LINK

ChromaX® A20
Affinity Chromatography Column
Product Manual



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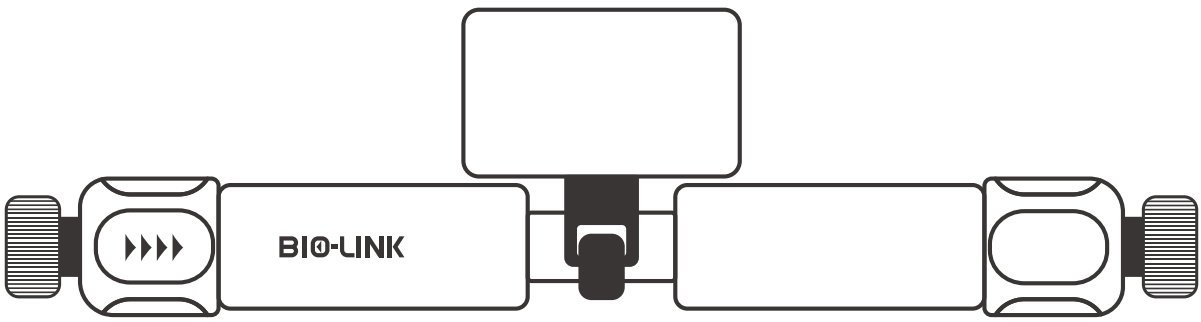
Chromatography Column Introduction

ChromaX®A20 is a protein A affinity chromatography column designed to quickly and accurately determine the antibody concentration (or titer) in cell culture supernatant, which is developed by unique surface treatment technique and coupling technique using recombinant protein A as ligand and polystyrene divinylbenzene (PS-DVB) microspheres with a porous structure as filling carrier.



Characteristics of ChromaX®A20 affinity chromatography column:

(1) Hydrophilic PS-DVB substrate, with the features of good rigidity, low back pressure, and fast mass transfer.

(2) Unique hydrophilic treatment technology with low non-specific adsorption ensures the accuracy of antibody concentration test results.

(3) Ligand specific binding sites, high selectivity ensures the accuracy of concentration determination results.

(4) Stainless steel column for direct use on HPLC.

(5) Fast and efficient, taking only 2 - 5 minutes to complete an analysis.

(6) Good linearity within the wide range of antibody concentration of 0.02 to 10 mg/mL

(7) Adopting alkali-tolerant protein A ligand allows for regenerating the chromatographic column with the 0.1 M NaOH solution.

Product Specifications

Table 1. Chromatography Column Specifications

Name	ChromaX®A20
Column specifications	ID2.1*30 mm; CV=0.1 mL
Matrix	PS-DVB
Ligand	Recombinant protein A
Mean particle size	20 μm
Dynamic binding capacity	> 20 mg hIgG / mL chromatography media*
Recommended flow velocity	0.5~2.5 mL/min
Max. Pressure resistance	< 1000 psi
PH stability range	pH 2 - 10 (common); pH 2 - 13 (CIP)
CIP cleaning agent	0.1 M NaOH, etc.
Operating temperature	4-40 ℃

*Test conditions for dynamic binding capacity: retention for 1 min, when the penetration amount of IgG reaches 10%, IgG loading amount (mg) of unit media volume (mL).

Instructions for Use

Preparation of buffer

The following buffers are recommended:

(1) Common starting buffer:
20-50 mM phosphate group, containing 0.15 M NaCl, pH 7.0-7.5

(2) Recommended elution buffer:
0.1% (v/v) hydrochloric acid (12 mM), containing 0.15M NaCl, pH 1.9, and acidic buffers (such as glycine, citrate, acetate, pH 2-3) can be applied in accordance with customer requirements for protein stability.

The water and reagents used for buffer preparation must be of high purity and filtered through 0.22 μm membrane before use.

Preparation of sample

(1) The recommended injection volume is 10-100 μL.

(2) Before injection, the sample needs to be centrifuged and filtered through 0.22 μm membrane.

(3) When the sample concentration is low, the injection volume can be increased.

(4) When the sample concentration is high, it is recommended to dilute the sample appropriately with the starting buffer.

(5) When the sample contains substances such as surfactants (e.g., Tween20 TritonX-100, etc.), polysaccharides, lipids, please consult the Technical Service Department.

Analysis steps

(1) Remove the plug screws at both ends of the chromatographic column, insert it to the HPLC system in the direction indicated on the column, and avoid air entering the column.

(2) Rinse out the preservation solution from the chromatographic column using 5-10 CV of pure water or starting buffer.

(3) To reduce the impact on the analysis by the baseline changes and disturbances generated at buffer switching, it is recommended to run the blank procedure for 3 - 5 times before normal injection, i.e., rinse the chromatographic column alternately with starting buffer and elution buffer, with 20-30 CV per stage.

(4) The starting buffer equilibrates the chromatographic column, with flow velocity of 30-50 CV 1-3 mL/min.

(5) Inject the sample.

(6) Let the 10-15 CV starting buffer pass through the chromatographic column.

(7) Elute by using 20-30 CV elution buffer.

(8) Rinse the chromatographic column 20-30 CV using the starting buffer.

Note: It shall not exceed the Max. back pressure of the chromatographic column.

Analysis method setting reference

Analytical column	ChromaX®A20（ID 2.1*30 mm）		
Flow velocity	1 mL/min，1800 cm/h		
Sample injection	20 μL sample to be tested		
Testing	UV 280 nm		
Equilibration/ rinsing solution	20 mM PB+150 mM NaCl, pH 7.2		
Eluent (select one of the solutions)	100 mM citrate, pH 2.5		
	100 mM acetate, pH 2.5		
	100 mM phosphate, pH 2.5		
	100 mM glycine, pH 2.5		
	0.1% HCl, 150 mM NaCl, pH 1.9		
Gradient	Time (min)	Gradient (%B)	Stage
	0.00	0	Equilibrate/rinse for 1 min, 10 CV
	1.00	0	
	1.01	100	Elute for 2 min, 、 20 CV
	3.00	100	
	3.01	0	Re-equilibrate for 2 min, 20 CV
		0	

Cleaning and Regeneration

ChromaX®A20 chromatography column shows a long service life by strictly following the instructions for operation. The chromatography column may cause phenomena such as increase in column pressure, decrease in recovery rate and load capacity due to the deposition of samples or miscellaneous proteins, irreversible adsorption of lipids, and other contamination. Regularly cleaning and regenerating the chromatographic column can effectively avoid increasing column pressure and removing residual impurities in the packing, thus achieving the goal of extending the service life of the chromatographic column.

Solvents that can be used for ChromaX®A20 chromatography column include 0.1 M NaOH solution, 1 M acetic acid solution, 1 M acetic acid solution containing 20% ethanol, 20% isopropanol solution, and elution buffer containing 1-2 M NaCl.

Recommended cleaning operation process:

(1) Reverse the chromatographic column into the HPLC system.

(2) Inject the cleaning solvent 2-3 times with the injection volume of 100 μL, (or, rinse the chromatographic column 5-10 CV with the cleaning solvent at the flow velocity of 0.1 mL/min).

(3) Rinse the chromatographic column with the starting buffer at the flow velocity of 0.5-1.0 mL/min.

(4) Insert the chromatographic column forward into the HPLC system.

Reminder: It is recommended to apply reverse rinsing to drive away the particles in the sieve plate of the column head and also to protect the lower half of the column bed from being contaminated. The cleaning solvent needs to be filtered through 0.22 μm membrane before use.

Storage

It is recommended to keep it in cold storage at 2 - 8 ℃ after rinsing the chromatography column (20 CV) with the starting buffer. No freezing!

Ensure to tighten the plug screws at both ends of the chromatography column to avoid column drying and cracking!

Ordering Information

Product name	Product code	Column tube material	Internal diameter × length	Column volume	Particle size
ChromaX®A20	2821-1111	SS	2.1×30 mm	0.1 mL	20 μm

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About Bio-Link

Bio-Link is a group of technology-driven businesses that provide process solutions in the life sciences industry. The company focuses on the development and production of the key processing equipment and consumables used in the manufacturing process of recombinant protein drugs, vaccines, antibodies, cell therapies, gene therapies, and other biological products. Bio-Link's portfolio of offerings covers the entire upstream and downstream bioprocess such as cell culture, single-use mixing and storage, chromatography, filtration (ultrafiltration/diafiltration, clarification, and virus removal), and hydration products, as well as process development services. Bio-Link is committed to providing customers with high-quality, innovative products and solutions and strives to build an efficient, safe and competitive biopharmaceutical supply chain eco-system.

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