BIOEAST

BiogCap® Protein A

Affinity Chromatography Media

BiogCap® Protein G

Affinity Chromatography Medium

Serve Science, See The Future

Hangzhou Bioeast Biotech Co., Ltd

COMPANY INTRODUCTION

HANGZHOU BIOEAST BIOTECH Co., Ltd. is a leading high-tech enterprise dedicated to pioneering research and development in the field of key raw materials for In-Vitro Diagnostic, Life Science, and Biomedical reagent solutions. Established in 2018, BIOEAST BIOTECH has emerged as a trusted provider of innovative solutions in the industry.

We specialize in developing advanced technologies and cutting-edge solutions to meet the evolving needs of the diagnostic, life science, and biomedical sectors. Our product portfolio encompasses a wide range of offerings, including microspheres, antigens, antibodies, enzymes, active proteins, chromatographic products, and integrated solutions for various diagnostic platforms such as Biochemistry, CLIA, ELISA, FIA, Rapid Test and Immunoturbidimetric assay.

With our leading R&D and manufacturing facilities, ISO13485 certified quality system, and round-the-clock technical support with assay development experience;

BIOEAST has proudly served hundreds of manufacturers with customized solutions and also has 2 wholly-owned companies to serve manufacturers for different needs. AICHEK - POCT integrated solutions BiogenMicro - Biomedicine raw material solutions

As your trusted partner along the way of life science, we are committed to creating a glorious future together. Let's collaborate to achieve breakthroughs and drive innovation in the industry.

BiogCap® Protein A Affinity Chromatography Media

Product Description

The product is composed of the new highly rigid agarose matrix with the independent intellectual property right and rProtein A ligands.

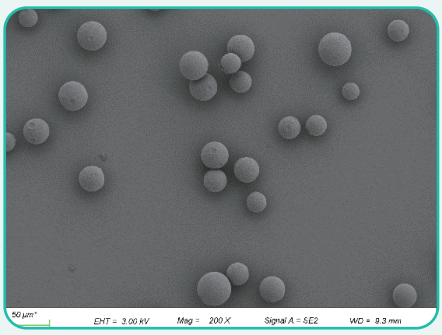
rProtein A is obtained through expression in Escherichia coli and purification and the production process is free from the risk of materials of animal origin. Compared with natural Protein A, the newly designed rProtein A features good alkali resistance and a stronger specific binding ability with IgG Fc fragments. The new, highly rigid agarose matrix is characterized by good chemical stability and biocompatibility. The fixed-point coupling technology is used to fix rProtein A on the highly rigid agarose matrix and thereby ensure that it retains the advantages of high capacity, high flow rate, good physiochemical stability, low nonspecific adsorption, little ligand falling, and a long service life. This product can satisfy the process requirements by further purifying the samples, including ascites, serum, and cell-culture fluid, so as to obtain highly pure antibodies. Therefore, it's an ideal choice for the large-scale production of antibodies.

Technical Parameters

See Table 1 for technical parameters of the product and see Figure 1 for the scanning electron microscope image.

Parameter	Index
Matrix	Highly rigid agarose
Ligand	Alkali-resistant rProtein A
Mean particle size	70 μm
Dynamic capacity (holding time of 6 min.)	65-80 mg h-IgG/mL wet gel
Maximum flow rate (25☒°C)	300 cm/h
Ligand falling	＜10 ng/mL
Maximum withstand voltage	0.3 MPa (3 bar)
Cleaning in place	0.1-0.5 M NaOH solution pH 2-12
Storage conditions	4-8°C (in 20% ethyl alcohol) Or (in 2% benzyl alcohol)

Table 1 Technical Parameters of BiogCap® rProtein A



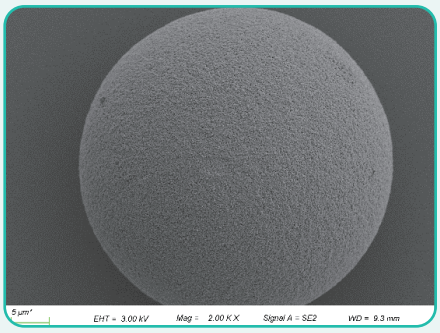


Figure 1: Scanning Electron Microscope Image of BiogCap® rProtein A

Capacity Test

Monoclonal antibody cell culture supernatant

Holding time: 6 min.

10% dynamic penetration capacity: 75 mg/mL

See the following figure for the chromatogram of measurement.

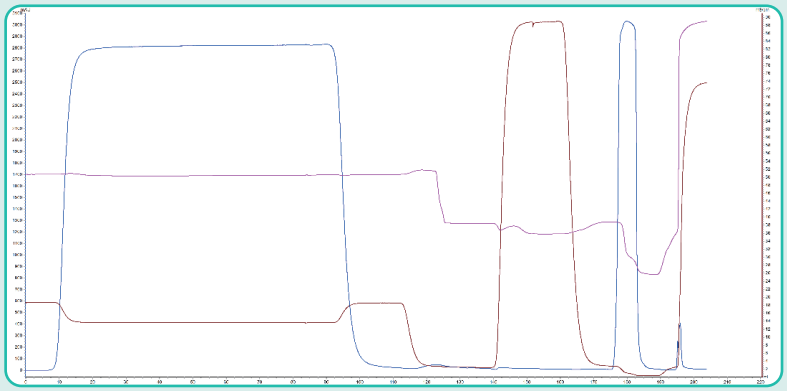


Figure 2: 10% Dynamic Penetration Capacity Chromatogram of BiogCap®rProtein A

The comparative data (with the same monoclonal antibody cell culture supernatant as mentioned above, the same experimental conditions and a product of the same type from another manufacturer) shows that the capacity of BiogCap®rProtein A is similar to that of the proteoglycan protein A packing of a foreign imported brand, as shown in the

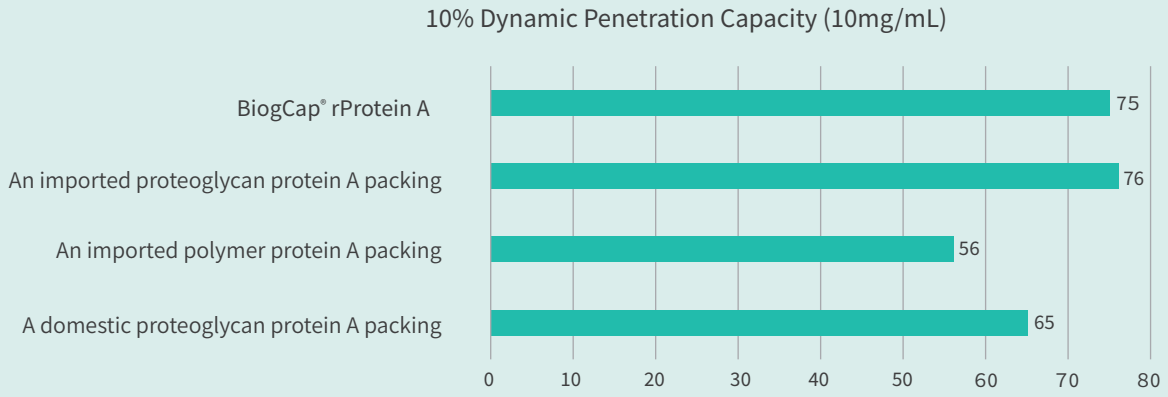


Figure 3: Schematic Diagram of Dynamic Capacity Comparison for BiogCap®rProtein A Packing

Statistic alkali resistance test

The comparative data (with the same monoclonal antibody cell culture supernatant as mentioned above, the same experimental conditions and a product of the same type from another manufacturer) shows that the capacity of BiogCap®rProtein A is similar to that of the proteoglycan protein A packing of a foreign imported brand, as shown in the

DBC (10%) before immersion	DBC (10%) after immersion
75 mg/mL	73 mg/mL

Table 2: Alkali Resistance Test Data

Pressure-flow rate curve

The recommended flow rate for BiogCap®rProtein A is 150 cm/h, and the maximum flow rate is 300 cm/h.

Experimental equipment: AKTA Pure 150

Experimental conditions: Packing height: 15 cm; column volume: 80 mL

Packing compression ratio: 1.1:1; temperature: 20°C; medium: water

Column model: XK26

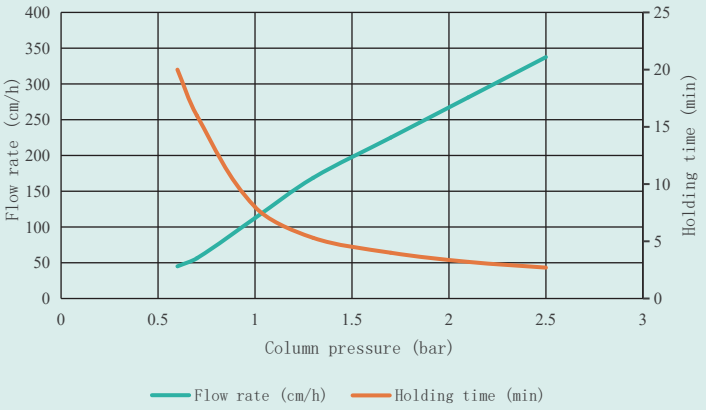


Figure 4: Pressure - flow rate Curve of BiogCap®rProtein A

How to use

Because the product has varying degrees of affinity to antibodies of different species and subspecies, and because the buffer conditions needed to sustain the bioactivity of different antibodies are different, it's recommended that users screen for the most appropriate method.

The following process is provided for the purpose of reference:

Equilibrium Use 5-10 CV of buffer A (20 mM PB+0.15 M NaCl, pH 7.0); or 0.05 M boric acid, 4.0 M NaCl, pH9.0) for column equilibrium (to the baseline).

Loading Load an appropriate number of samples (using buffer A to prepare solid samples and use buffer A to dialyze the liquid samples).

Washing Use 5 CV of buffer A to wash the column (to the baseline).

Elution Use buffer B (20 mM citric acid, pH 3.0; or 0.1 M glycine, pH 3.0; or 20 mM sodium acetate, pH 3.0) for 10 CV linear elution, till 100% B is attained.

Neutralization Collect the elution products, use neutralizing buffer solution (1.0 M Tris-HCl, pH 9.0, or other) to dilute the antibody till a stable pH value is attained.

Reproduction After being used a certain number of times, the column should be reproduced and then cleaned in place. The following two methods are provided:

(1) Apply 0.1 M acetic acid or 0.1 M acetic acid/20% ethyl alcohol to drip wash the column for 3-5 CV, and then use buffer solution to wash and neutralize it for the next use.

(2) Apply 0.1-0.5 M NaOH to drip-wash the column for 3-5 CV. Use 3-10 CV of pure water for rinsing, and then use buffer solution to wash and neutralize it prior to reuse.

List of Products

Cargo No.	Description	Specifications
AF07-0001	BiogCap®rProtein A	10 mL
AF07-0005		50 mL
AF07-0025		250 mL
AF07-0050		500 mL
AF07-0100		1L
AF07-P001		1 mL prepacked column
AF07-P005		5 mL prepacked column

BiogCap® Protein A Affinity Chromatography Media

Product Description

This product is manufactured with recombinant Protein G as a ligand and new high-rigidity agarose beads as a matrix. Protein G can be used for the purification of antibodies (monoclonal and polyclonal) by specifically binding to the fc fragment of the antibodies. This product can obtain high-purity antibodies from animal ascites, serum and culture medium through one-step purification. Given the antibody binding facilitated by the Fab segment, the Protein G affinity chromatography medium is more suitable for the separation of immune complexes. Additionally, BiogCap® Protein G has the advantages of high load, good physical and chemical stability, long service life and a ligand that doesn't fall off easily.

Protein G has different binding properties with respect to IgG compared than Protein A has. The distinction is primarily manifested in the fact that Protein G binds more readily to polyclonal antibodies.

Product Parameters

Table 1 Technical Parameters of BiogCap® Protein G

Parameters	Indicators
Matrix	High-rigidity agarose
Ligand	Protein G
Shape	Sphere
Average particle size	75 μm
Dynamic load	Not less than 35 mg h-IgG/mL wet gel
Maximum flow rate (25°C)	450 cm/h
Recommended flow rate	150 cm/h
Operating pH	3-9
In-place cleaning pH	2-10

Product Morphology

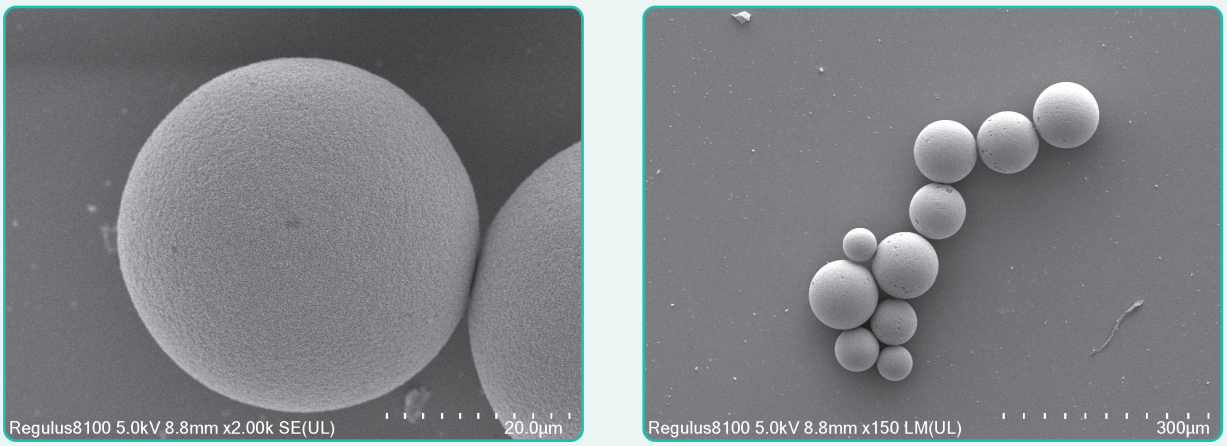


Figure 1 Electron Microscope Photographs of BiogCap® Protein G

Product Description

Protein A packing material, with its early development history, shows excellent performance. For example, BiogCap® rProtein A has been able to achieve a dynamic load greater than 75 mg/mL for human IgG. Though slightly poor in load performance, BiogCap® Protein G can bind to more types of antibodies in a broader, stronger manner. Thus, BiogCap® Protein G can be used together with BiogCap® rProtein A.

Table 2 Comparison of Binding Capacity between Common Antibodies and Protein A/G

Species	Subclass	Protein G binding	Protein A binding
Human	IgA	-	Variable
	IgD	-	-
	IgE	-	-
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	++++	-
	IgG4	++++	++++
	IgM	-	Variable
Avian egg yolk	IgY	-	-
Cow		++++	++
Dog		+	++
Goat		++	-
Camel		+	-
Mouse	IgG1	++++	+
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	+++	++
	IgM	-	Variable
Pig		+++	+++
Rabbit		+++	++++
Rat	IgG1	+	-
	IgG2a	++++	-
	IgG2b	++	-
	IgG3	++	+
Sheep		++	+/-

Load Test

Sheep antibody is often purified by Protein G column due to its poor affinity with Protein A. We used BiogCap® Protein G to test the load of sheep antibody, and its 10% penetration load was approximately 34 mg/mL. The test map is as follows:

Experimental instrument: AKTA

column diameter: 6.6 mm

column height: 9.7 cm

flow rate: 200 cm/h

retention time: 3 min.

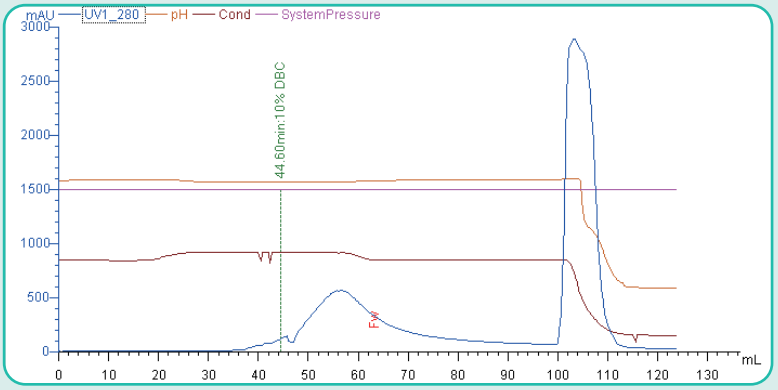


Figure 2: Dynamic Load Map of BiogCap® Protein G @ Sheep IgG

Additionally, for reference we tested the load of BiogCap® rProtein A and BiogCap® Protein G for different antibodies, as shown below:

Table 3 10% Penetration Load of BiogCap® rProtein A and BiogCap® Protein G for Different Antibodies

		BiogCap® rProtein A	BiogCap® Protein G
S/N	Antibody Type	Load (mg/mL)	Load (mg/mL)
1	Human IgG	79	45
2	Mouse IgG	57	16
3	Sheep IgG	-	34
4	Rabbit IgG	68	-
5	Goat anti-mouse IgG	24	-
6	Cow IgG	55	-

Pressure Flow Rate Curve

The recommended flow rate of BiogCap® Protein G is 150 cm/h, and the maximum flow rate exceeds 450 cm/h.

Experimental instrument: AKTA
Experimental conditions: Column packing
height: 20 cm
column volume: 40mL
column packing compression ratio: 1.15:1
Temperature: 20°C
medium: water
Column model: XK16

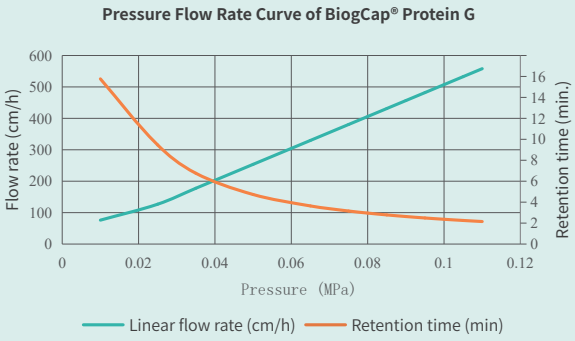


Figure 3 Pressure Flow Rate Curve of BiogCap® Protein G

Denaturant-Resistant Performance

BiogCap® Protein G must be regenerated after reaching a certain number of uses before it can be used again. The packing material's tolerance to the protein denaturant is among the key factors that will affect the service life of the packing material. We used 2 CV 6 M guanidine hydrochloride and 3 CV deionized water for alternate cleaning for 100 cycles, after which we compared the changes in dynamic load before and after cleaning. The results are shown below:

Table 4 Load Change of BiogCap® Protein G

	Load percentage
Before cycle	100%
After cycle	92.5%

The results showed that BiogCap® Protein G had good tolerance to 6 M guanidine hydrochloride.

Application Method

This product has different degrees of affinity with antibodies of different species and subtypes, and different buffer conditions are necessary in order to maintain the biological activity of different antibodies. It is therefore recommended that method screening be performed.

A reference process is provided:

Equilibrium

Use 10 CV of buffer A (20 mM PB+0.15 M NaCl, pH 7.0) for column equilibrium (equilibrium to the baseline).

Loading

Load an appropriate amount of the sample. (Solid samples can be prepared with buffer A, while liquid samples can be dialyzed with buffer A.)

Washing

Use 5 CV of buffer A to wash the column (rinsing to the baseline).

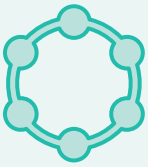
Elution

Use buffer B (20 mM citric acid, pH 3.0 or 0.1 M glycine, pH 3.0 or 20 mM sodium acetate, pH 3.0) for 10 CV linear elution until 100% B. (For antibodies with strong binding capacity a lower pH, such as pH 2.5, can be used for elution.)

Neutralization

Collect the elution products and use an alkaline buffer (1.0 M Tris-HCl, pH 9.0 or other) to dilute the antibody to a stable pH value.

Neutralization



After a certain number of uses, the column must be regenerated and cleaned in place. The two methods are provided below:

(1) Use 0.1 M acetic acid or 0.1 M acetic acid/20% ethanol to wash column 3-5 CV and then use buffer solution to wash them till they' re neutral for reuse.

(2) Additionally, use 70% ethanol or 6 M guanidine hydrochloride to wash column 2-4 CV, flush them with 3-10 CV pure water and then use buffer solution to wash them till they' re neutral for reuse.

BIOEAST

Hangzhou Bioeast Biotech Co., Ltd.

Room 701&702,Building #3,Hexiang Technology Center
Qiantang New Area 310018 Hangzhou,Zhejiang Prov,China

international@bioeast.com

+86 190 1283 0538

en.bioeast.com