AGAROSE BEAD TECHNOLOGIES

APPLICATION NOTE

PROTEIN G AGAROSE 4 RAPID RUN™ AFFINITY CHROMATOGRAPHY RESIN

BENCHMARKING STUDIES

Introduction

Protein G is a well-established ligand for affinity chromatography resins used for isolation and characterization of immunoglobulins. Protein G binds to the Fc region of mammalian IgGs thus exhibiting high affinity for many IgG subclasses including human, rabbit, mouse, rat, sheep, pig, horse, cow or goat. In contrast to protein A, protein G binds all human and mouse IgG subclasses, including human IgG3 and mouse IgG1 as well as all rat IgG subclasses. Furthermore, protein G also binds to CH1 portion of the Fab region making this ligand well suited for purification of more complex constructs such as bispecific antibodies.

Product introduction

Protein G Agarose 4 Rapid Run affinity chromatography resin was developed by Agarose Bead Technologies for purification of immunoglobulins. It is characterized by improved selectivity, high binding capacities and drastically improved flow properties resulting in very high productivities even in large scale columns. The resin is suited for purification of a broad range of IgG species and subclasses both at the small and large scales.

Protein G Rapid Run resin is part of ABT agarose-based resins range for purification of biological products, which ensures security of supply and comprehensive technical and regulatory support. General characteristics of protein G Agarose 4 Rapid Run are shown in Table 1.

PRODUCT NAME	PROTEIN G AGAROSE RESIN 4 RAPID RUN™
Cat. No.	4RRPG-X
Bead Geometry & Size	Spherical, Standard: 50-150 µm
Crosslinked	Highly crosslinked
Agarose %	4%
Coupling Method	Coupling binding by reductive amination
Static Binding Capacity	>20 mg human IgG/ml resin
Maximum Flow Rate*	≥ 900cm/h
Cleaning solution	0.1% Non-ionic detergent (e.g. Triton-X)
Sanitization	20% or 70% ethanol depending on contact time
Antimicrobial Agent	20% Ethanol
Storage Temperature	2-8° C

*Data analysed on the matrix 4% Rapid Run™ Agarose Bead Standard. Column: XK 16/40 bed height 15 cm. System: ÄKTA Purifier UPC 100. The highest flow that beads withstood for 1 minute without collapsing and the pressure reaching 1 MPa.

Table 1: General characteristics of Protein G Agarose Resin 4 Rapid Run™.

Benchmarking studies

In this application note, a head to head comparison of three commercially available protein G resins is presented. The comparison was performed by an independent CRO laboratory with a high expertise in antibody purification. The study focused on comparison of binding capacities, yields and purities for targets purified from different real feed samples. The targets included, two monoclonal and two polyclonal antibodies from different sources. All three resins used in this study were agarose based. This choice allowed for the comparison to be based solely on ligand type, coupling chemistry and base bead morphology, as the non-specific binding to the agarose base matrices should in principal be the same for all the resins. In addition to different chromatographic characteristic, the resins also differ from a commercial perspective such as price per liter and security of supply, including the geographical location of the resin manufacturing site.

Materials

The basic chromatographic and commercial attributes of the three resins are listed in Table 2. Since the resins differ in the list price per liter, a normalized price per liter was also included as one of the attributes to describe the resins from the commercial perspective.

	Protein G Agarose 4 Rapid Run™	Protein G Sepharose™ 4 Fast Flow	Protein G Resin FF
Manufacturer	ABT (EU)	Cytiva (EU)	Genscript (ASIA)
Matrix	4% highly cross-linked agarose. Average particle size 90 µm (50-150 µm)	4% highly cross-linked agarose. Average particle size ~ 90 µm	4% highly cross-linked agarose. Average particle size 90 µm (45-165 µm)
Ligand	Recombinant Streptococcal protein G lacking the albumin-binding sites expressed in E. coli. MW 21,6KDa, pI 4.1	Recombinant protein G lacking the albumin-binding sites expressed in E. coli. MW 17KDa, pI 4.4	Recombinant Streptococcal protein G lacking the albumin-binding sites expressed in E. coli. MW 22KDa, pI 4.69
Properties of the resin	Binding capacity > 20mg human IgG/ml of resin	Binding capacity > 20mg human IgG/ml of resin	Binding capacity > 20mg human IgG/ml of resin
Relative Price	€€	€€€	€€

Table 2: Chromatographic and commercial attributes of the three tested resins.

The information about the type, source, method of production and the specific antigen to detect a given target(s) in the flow through fractions used in the study is provided in Table 3.

Type	Source	Production method	Antigen*
IgG1	Murine hybridoma Specific antibody against human Fc region	Grown in a bioreactor with culture medium ClonaCell HY-MediumE (high cell density and high content of fetal bovine serum (FBS)	Chimeric Ephrin-Fc protein containing human Fc expressed in mammalian CHO cells
IgG2a	Murine hybridoma Specific antibody against human Anexin 4 (ANXA4) protein	Grown in a flask with RPMI culture medium (low cell density and moderate content of FBS).	Recombinant Anexin 4 expressed in mammalian cells HEK293
Goat polyclonal	Serum	Goats with a known reactivity for the p22 protein	P22 protein
Rabbit polyclonal	Serum	Rabbit immunized against human EDIL3 protein	Recombinant EDIL3 expressed in E. coli

Table 3: List of antibodies, their origins and methods of production, and the specific antigen used in the study.
* used for ELISA and Western Blotting.

Methods

Chromatography method

Conditions: 1mL column EB-CTG1 (Agarose Bead Technologies), flow rate 1mL/min, binding and washing buffer: 25 mM phosphate buffer pH 7.0; elution buffer: 0.1 M glycine pH 2.8. Sample as per text in the respective panel.

SDS/PAGE

Feed, Flow Through (unbound fraction), and elution fractions (Pool A with 70% of eluted IgG and Pool B with 30% of eluted) were collected and loaded onto a 10% acrylamide gel.

To quantify the IgG concentration in Pool A plus Pool B, both pools were loaded onto a 10% acrylamide gel. ImageJ software was used for quantification by band densitometry using a standard curve prepared with BSA (range 100 to 700 ng per well).

To confirm the results, a second quantification was performed measuring the IgG pools by NanoDrop™ analysis (abs 280 nm).

ELISA

Plates were coated and incubated overnight at 4 ºC with an antigen with known reactivity against each type of antibody, as shown in Table 3.

Washing step: 3x with PBS + 0.05% Tween (PBST). Plate blocking step: 200 µl of PBST solution + 3% skimmed milk powder per well.

The purified IgGs were added in serial dilutions with PBST + 3% milk, incubated 1 hour at RT.

The secondary antibodies chosen IgG-HRP were added and diluted at 1:5000 in PBST + 3% milk, incubated 1 hour at RT.

Finally, the chromogenic substrates were added: TMB and then 1 M HCl to stop the reaction. Absorbance measured at 450 nm.

Results

Chromatograms comparing the three resins for each of the feeds considered in this study are presented in Figure 1. Respective SDS-Page gels are shown in Figure 2.

Both, the breakthrough curves shape and the size of elution peaks shown in Figure 1, clearly show that binding capacities for ABT resin are much higher as compared to the Genscript resin and are the same or slightly higher as compared to the Cytiva resin.

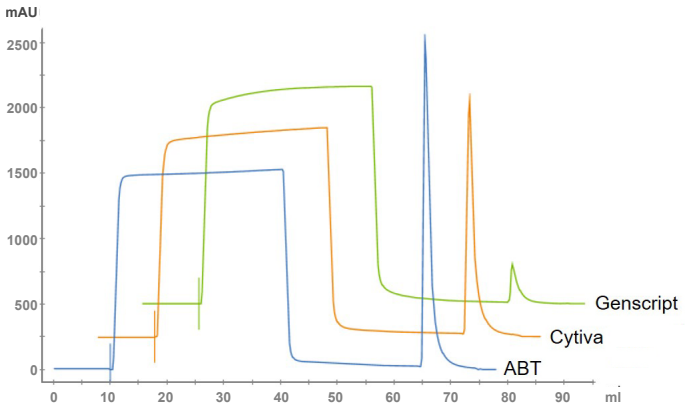
Purities of the eluted IgGs are similar for all the resins (Figure 2), and are independent on the amount adsorbed.

The relative amounts either eluted from each of the columns or found in the flow through fractions are shown in Figure 3A and 3B, respectively.

These data, obtained using two orthogonal detection methods, confirm the conclusion drawn from Figure 1 regarding the binding capacities for the three resins tested (i.e., the resin with a higher binding capacity will have a lower amount of the target IgG found in the flow through). The data in Figure 3 is expressed as relative to the data obtained for the ABT resin.

A)

Early breakthrough clearly seen for Genscript and Cytiva resins, indicate that the binding capacities for these resins are lower tan for the ABT resin.

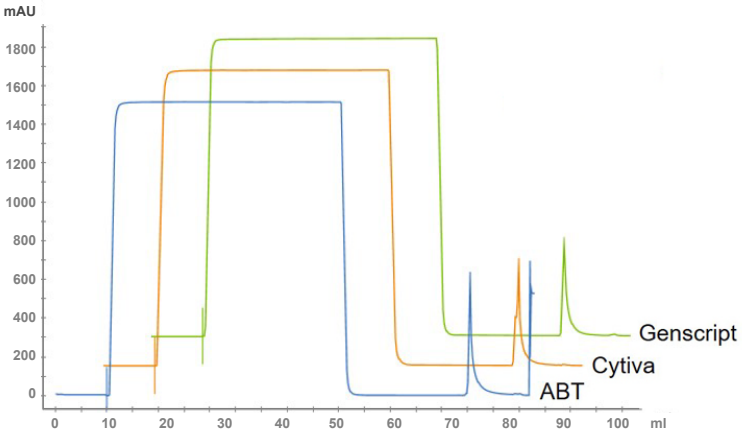


The size of elution peak is proportional to the amount bound and retained on the column post the wash step:

ABT > Cytiva >> Genscript

B)

No visible breakthrough for any of the resins indicate that none of the resins was loaded beyond its binding capacity.

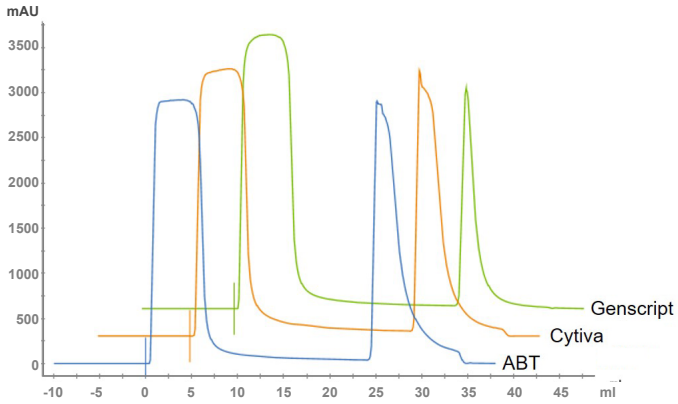


Size of the elution peaks confirms similar binding capacities for all three resins:

ABT = Cytiva = Genscript

C)

Early breakthrough for the Genscript and Cytiva resins indicates lower binding capacities as compared to the ABT resin.

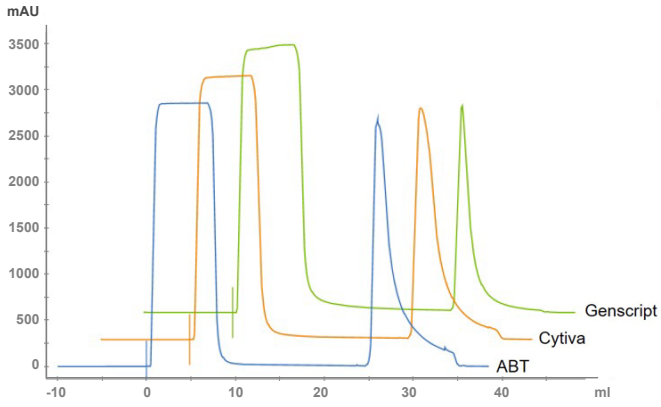


Size of elution peaks shows different capacity levels for all three resnis:

ABT > Cytiva > Genscript

D)

Early breakthrough for the Genscript resin indicates lower binding capacities as compared to the ABT and Cytiva resins



Size of elution peaks shows different capacity levels of Genscript resins:

ABT = Cytiva > Genscript

Figure 1: Chromatograms from a one-step purification of A) IgG1; B) IgG2a; C) polyclonal goat antibodies; D) rabbit polyclonal antibodies on three agarose-based protein G affinity resins: Agarose Bead Technologies Protein G 4 Rapid Run™ (blue curve), Cytiva Protein G Sepharose™ 4 Fast Flow (yellow curve), and Genscript Protein G FF Resin (green curve).

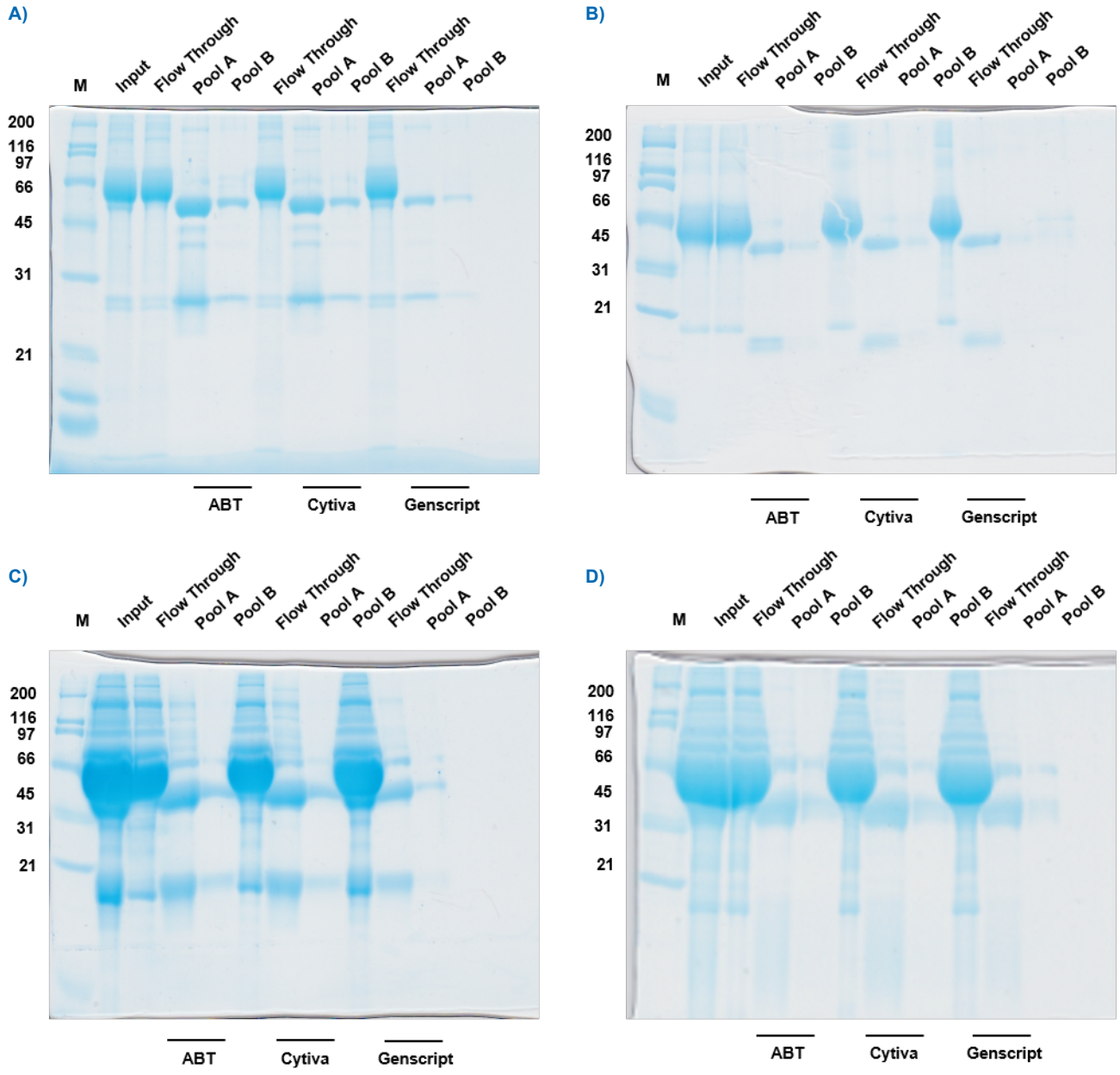
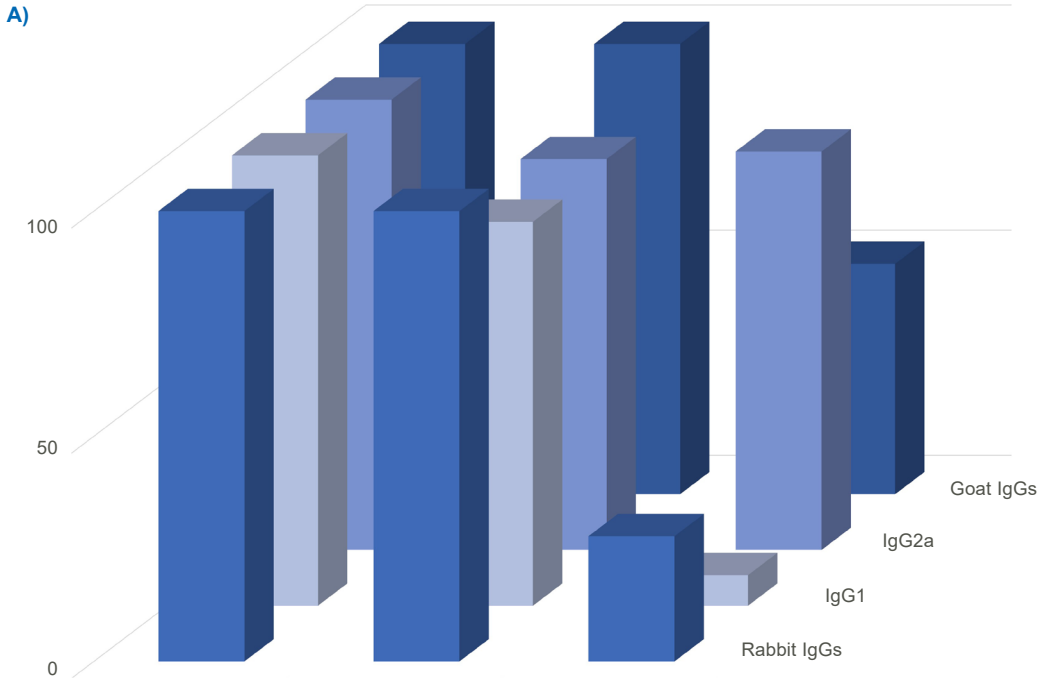
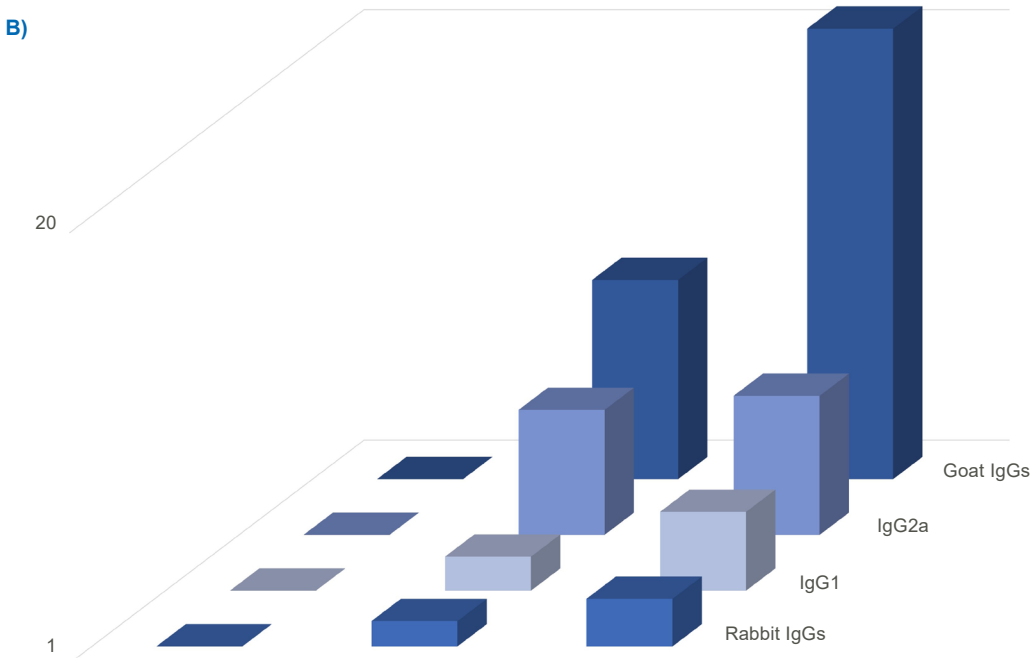


Figure 2: SDS-PAGE of non-reduced samples of fractions collected in experiments shown in the respective panels in Figure 1. Lanes: M - Molecular Weight Calibration Kit; Input – load sample, Flow Through - unbound fraction; Pool A – first 70% of elution pool; Pool B – remaining 30% of elution pool.



	ABT	Cytiva	Genscript
Rabbit IgGs	100	101	28
IgG1	100	85	7
IgG2a	100	87	88
Goat IgGs	100	100	51



	ABT	Cytiva	Genscript
Rabbit IgGs	1,0	1,2	1,4
IgG1	1,0	1,3	1,7
IgG2a	1,0	2,4	2,6
Goat IgGs	1,0	4,0	23,0

Figure 3: Chromatographic performance attributes of protein G resins evaluated in the benchmarking study describing purification of different antibodies from real feed samples: A) amount of IgG captured by resin based on densitometry data expressed in percent of the amount captured by ABT resin; B) amount of target found in the Flow Through fraction measured using antigen ELISA normalized by the results obtained with ABT resin. The signal in the Flow Through determines if the antibodies were lost due to their inability to recognize their target. ABT showed a better recovery in all cases as determined by the decreased signal.

Conclusions

The Protein G Agarose 4 Rapid Run affinity resin manufactured by Agarose Bead Technologies is a product that has the best quality cost balance among the three resins tested in this study, presenting great characteristics in terms of yield, stability and selectivity.

Combination of the unique selectivity of the modified protein G ligand, and the optimized properties of Rapid Run agarose base beads makes the Protein G Agarose 4 Rapid Run affinity chromatography resin the leading resin for both the laboratory as well as industrial scale applications.

About Agarose Bead Technologies (ABT)

Agarose Bead Technologies has been providing solutions to the biopharmaceutical industry for over 20 years for the separation, immobilization and purification of biomolecules like recombinant proteins, enzymes, peptides, antibodies, etc.

ABT offers a wide range of non-activated agarose resins for Size Exclusion and activation procedures, as well as activated agarose beads for Affinity Chromatography, Ion Exchange Chromatography, and Pre-activated resins for coupling of affinity ligands (immobilization).

As part of pharmaceutical development chain, ABT embodies an unwavering commitment to the quality of its products and services, at every step of the way. ABT aims to meet quality and regulatory standards in order to exceed customer expectations in all aspects of product supply.

ABT is an ISO 9001:2015 certified company and has two well established manufacturing plants in compliance with Good Manufacturing Practices (cGMP).

Ordering information

PRODUCT NAME	CAT.	PACK SIZE
Protein G Agarose Resin 4 Rapid Run™	4RRPG-5	5 ml
	4RRPG-25	25 ml
	4RRPG-100	100 ml
	4RRPG-500	500 ml
Protein G Affinity Cartridges 5ml	AF4PG-Ctg5-1	1 x 5 ml
	AF4PG-Ctg5-5	5 x 5 ml
Protein G Test Kit	4RRPG-K01	100 µl
4% Rapid Run™	4RRS-500	500 ml
	4RRS-1000	1000 ml

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If you are interested in the customization of agarose resins, and you would like more information, contact us today at

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