MabSpeed™ rP202

MabSpeed™ rP202 (superior resistance) is a member of the MabSpeed™ family of affinity chromatography media for the capture of monoclonal antibodies (MAbs) at process scale. MabSpeed™ is composed of a rigid, high-flow methacrylate matrix with engineered type of protein A ligand chemically immobilized. This ligand provides greater stability than conventional wild type of protein A-based media under the alkaline conditions used in cleaning-in-place (CIP) protocols. In addition, MabSpeed™ rP202 offers superior binding capacity with fast kinetic enabling to offer improved overall process economy.

Figure1. MabSpeed™ series are manufactured in Fukuoka, Japan, with state-of-the-art technology and are available in both small and large scales.

Characteristics of MabSpeed™ rP202:

- Novel, alkali-stabilized protein A ligand allows the use of 0.1-0.5N sodium hydroxide for CIP.
- Improves product quality and reduces overall costs.
- Novel ligand design results in lower ligand leakage.
- Generic elution conditions for different monoclonal antibodies enables platform approach to purification.
- High dynamic binding capacity (DBC) reduces process time and amount of medium used.
- Fast kinetics, i.e. fast adsorption and desorption, leads shorter operation time.
- High mechanical resistance allow processing with high flow rate, up to 1,000 cm/h.

MabSpeed™ series:

The MabSpeed™ family of media for process-scale purification of monoclonal antibodies comprises MabSpeed™ rP102, rP111, and rP202. MabSpeed™ rP102 uses wild type of Protein A ligand and is designed for high-throughput purification of monoclonal antibodies from large volumes of feed with a particle size of 45 µm. For more information on MabSpeed™ rP102 and rP111, please refer to Data Sheet No. 03-07-C-0101. MabSpeed™ rP202 has been developed from the same rigid, highly cross-linked methacrylate materix used for MabSpeed™ rP102 and rP111. The matrix of MabSpeed™ rP202 allows the use of higher flow rates in process scale purification of Mabs compared with conventional agarose types of family with fast kinetics, suggesting that Mabs can be processed with high productivity and efficiency.

***Note:** MabSpeed™ rP202 media/columns are available in Asia and will be available in other regions in the end of 2016. For details, please inquire us online at www.diaion.com/en.

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MabSpeed™ rP202

High stability in alkaline conditions:

The MabSpeed™ rP202 ligand was developed by protein engineering of C-domain of the IgG-binding Protein A. Particularly sensitive part in amino acids were identified and substituted with more stable sequences. By engineering IgG-binding site of Protein A, binded IgG can be eluted at a mild condition around pH3.5. The final construct is a multimer of the engineered C-domain of Protein A with some specific C-terminal. The ligand is produced by fermentation and downstream processes and the entire production process is managed by ISO9001 and free of components of mammalian origin. The resulting ligand is chemically immobilized to the methacrylate matrix through a chemically stable immobilization technique.

Characteristics of MabSpeed™ rP202:

Grade name	MabSpeed™ rP202
Ligand	Alkali stabilized rProtein-A (engineered type)
Ligand coupling method	Chemically immobilized
Matrix	Rigid, highly cross-linked methacrylate
Average particle size	45 µm
Pore size*	≥ ~1000 Å
Dynamic binding capacity**	> 50 g/L-media
Chemical stability	Stable in all aqueous buffers commonly used in protein A chromatography
pH working range	1 to 12
Cleaning-in-place stability	0.1-0.5N NaOH
Temperature stability***	2°C-40°C
Max. mobile phase velocity	≤ 1,000 cm/h
Delivery conditions	20% ethanol

* Pore size listed above is referential data measured by mercury intrusion method.

** DBCs are measured with a human polyclonal IgG with a bed height: 5cm, a residence time of 6 min with a 10% break-through point.

*** Recommended long term storage conditions: +2°C to +8°C, 20% ethanol.

Hydraulic data represents low pressure drop and easy packing:

The spherical and mono-dispersed particles in nature offers easy packing for MabSpeed™. The hydraulic data is important in aspect of column packing. Figure 2 represents the hydraulic data for MabSpeed™ rP202 among competing products available on market. As shown, MabSpeed™ rP202 has a linear correlation with the linear velocity as theoretically predicted, while other products have exponential increase.

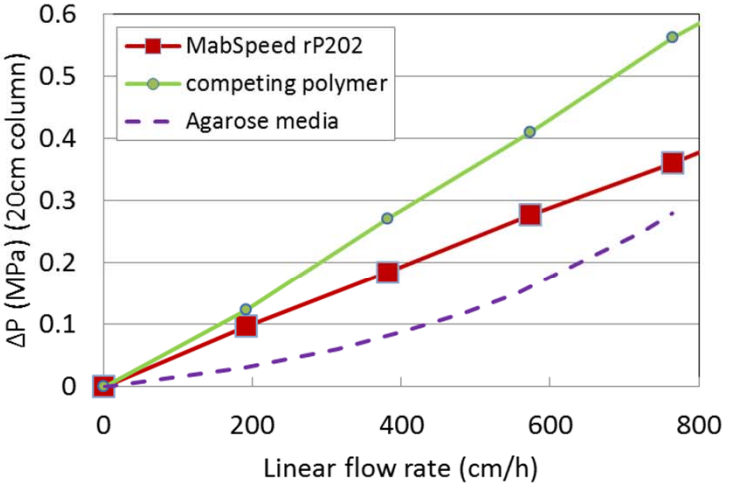

Figure 2. Pressure drop data. Data was taken with a condition of a column 20 mm ID x 200 mm with liquid water at temperature 25 °C.

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MabSpeed™ rP202

High dynamic binding capacity over a wide range of flow rate, r.t.:

High dynamic binding capacity (DBC) is an essential characteristics in the process of affinity chromatography. MabSpeed™ rP202 offers dynamic binding capacity, well exceeding those agarose and/ or polymeric media in standard/ traditional flow rates of 100 cm/h (r.t of 12 min) to 400 cm/h. In addition, by tuning the polymeric media with original technology, MabSpeed™ rP202 is able to keep the high dynamic binding capacity even at high flow rate in the range of 600 cm/h (r.t. of 2 min) to 1,000 cm/h (r.t. of 1.2 min), while those agarose media often face mechanical difficulties, such as high pressure drop at these conditions.

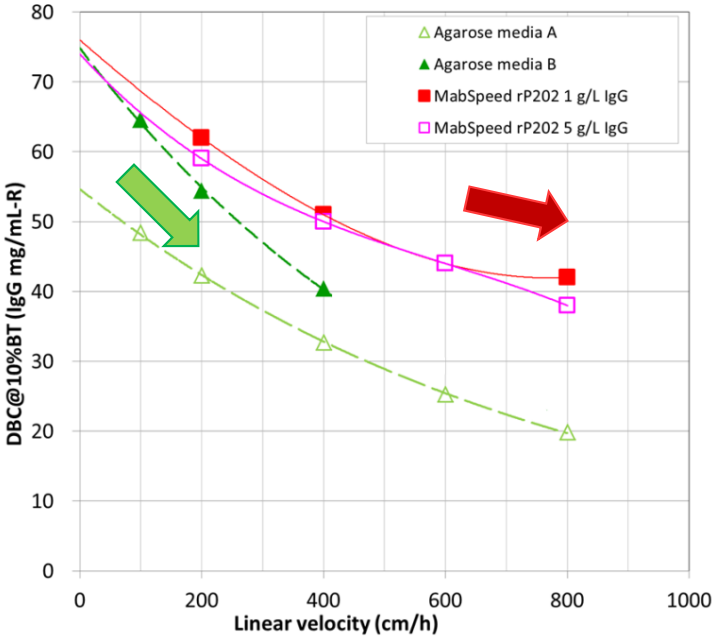

Figure 3. DBC profile curves with respect to a linear velocity in cm/h. Data was taken with a column 5 mm ID x 200 mm with a sample of 1 mg/mL human IgG. The straight red arrow shows high DBC at high flow rate while the blank green arrow of agarose media shows significant decrease of DBC.

High dynamic binding capacity and Protein-A ligand leakage after numerous CIP cycles:

In industrial applications, cleaning-in-place is an essential step in the production of pure MABs. One of the drawbacks using sodium hydroxide for CIP of conventional protein A-based media is the sensitivity of native and recombinant Protein A (rProtein A) to alkaline conditions. MabSpeed™ rP202, however, retains dynamic binding capacity after repeated CIP cycles with 0.5N NaOH. Figure 4 is an example of such performance. This represents that the DBC was kept stable even after 300 times of vigorous CIP with 0.5N NaOH, and the Protein A ligand leakage was kept under 10ppm.

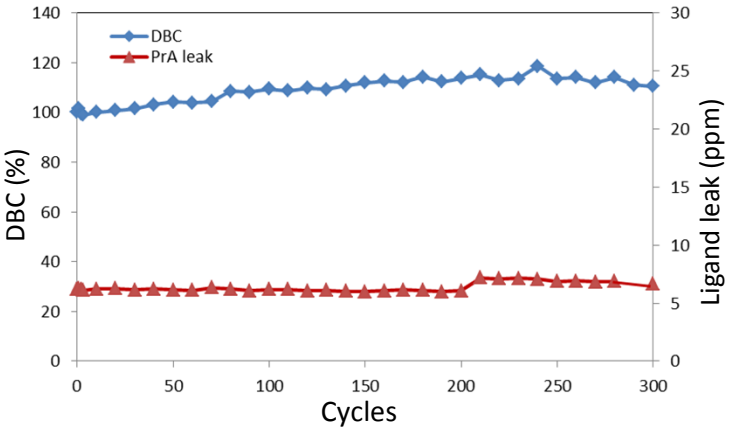

Figure 4. Dynamic binding capacity and Protein-A leakage after CIP with 0.5N NaOH. Experiments were performed with a column of 5 mm ID x 5 cm, by binding with phosphate buffered saline (pH 7.4) and eluting with 0.1M sodium citrate at pH 3.0. The contact time was 15 min.

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MabSpeed™ rP202

Low HCP contamination and Protein-A ligand leakage with high recovery with CHO cell culture purification:

One example of CHO cell culture purification is represented in Figure 5 with MabSpeed™ rP202. The data indicates sharp elution just like other media seen on market. Analysis of HCP contaminants and the recovery was measured. As listed in Table 1, the MabSpeed™ rP202 offers superior alternative for such purifications, with excellent performance of HCP contaminant removal of three log reduction.

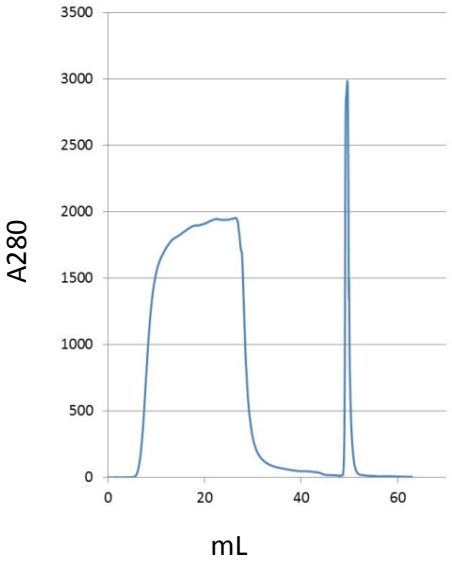

Figure 5. CHO cell culture (0.99mg/mL mAb, 30mL) purification with conditions of: a column (MabSpeed™ rP202, 7 mm x 26 mmH, binding buffer (PBS), wash buffer (PBS), elution buffer (0.1M citrate pH 3.0), flow rate (1.0 mL/min), with a system AKTA avant.

MabSpeed™ rP202 enables elution with weakly acidic condition of pH 4.0:

Our engineered ligand was designed to have both alkali tolerance and ability to elute with weakly acidic environment. Figure 6 is showing a purification of herceptin (mab1) and rituxan (mab2) on both MabSpeed™ rP111 and MabSpeed™ rP202. As shown, MabSpeed™ rP202 achieves purification of those two with eluate pool pH of 3.9, which was not previously able to do so with MabSpeed™ rP111.

Table 1. Analysis at each purification step of CHO cell culture, conducted with Figure 5.

Starting material		116,000 ppm (ng-HCP/mg-IgG)
IgG fraction	MabSpeed™ rP202	19 ppm(ng-HCP/mg-IgG) ⇒*>3 log reduction*
Recovery	MabSpeed™ rP202	>98% (UV280nm)

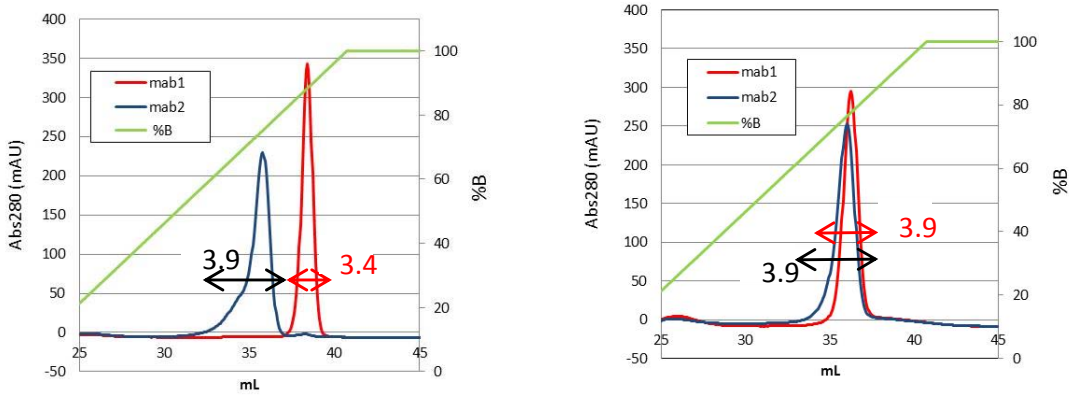

Figure 6. Elution profiles comparing MabSpeed™ rP111 (left, with wild type ligand) and MabSpeed™ rP202 (right, with *new* engineered ligand). Numbers in the profiles are pH of eluate pool. Data are taken with a condition of a column 5 mm ID x 50 mm, flow rate 1.0 mL/min, eluent A 0.1 M, citrate pH6.5, eluent B 0.1 M citrate pH2.5, pH gradient 0->100 %B over 20 CV (20 mL), and with a sample of 20 µg/mL mab in TST, 10 mL (0.2 mg).

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MabSpeed™ rP202

Fast kinetics leads to shorter operation time:

MabSpeed™ series brings you fast adsorption and fast desorption. For instance, Figure 7 shows that the desorption, i.e. buffer exchange profile, shows that the at each flow rate, MabSpeed™ offers faster processing, saving both time and buffer solutions of >10% compared with agarose resin.

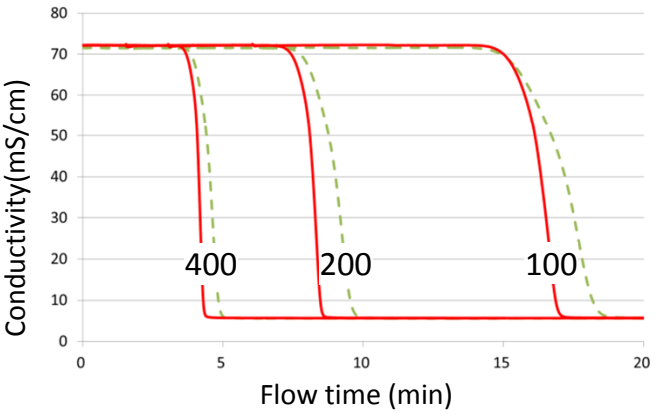

Figure 7. Buffer exchange profile at three flow rates of 100, 200, and 400 cm/h, comparing Mabspeed™ rP202 (solid red) and agarose media (dotted green). Experimental conditions: a column of 10mm ID x 200 mm, base of 0.8M NaCl, load with 0.05M NaCl, with a temperature of 20°C. Time to equilibration was measured by ~6.0mS/cm.

The shorter operation time is expected with MabSpeed™. A preliminary productivity simulation shown in Table 2. As seen on the table, MabSpeed™ rP202 offers higher throughput, i.e. IgG production per day throughout the range of 200 cm/h to 400 cm/h. MabSpeed™ additionally has advantages to run even faster flow rate of 600 cm/h, which would bring even higher productivity.

Table 1. Preliminary productivity simulation comparing MabSpeed™ rP202 and agarose media.

		MabSpeed™ rP202			Agarose resin A		Agarose resin B	
		200cm/h	400cm/h	600cm/h	200cm/h	400cm/h	200cm/h	400cm/h
min/ cycle	adsorption	372	153	88	254	98	326	121
	misc.	93	54	42	105	60	105	60
cycle/day		3.1	6.9	11.1	4.0	9.1	3.3	7.9
DBC (1% BTC)		***62***	***51***	***44***	*42*	*33*	*54*	*40*
Column volume (L)		1.57						
IgG production (g/day)		***301***	***555***	***769***	*267*	*468*	*285*	*504*

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Technical Data Sheet

MabSpeed™ rP202

Ordering information:

Product Name/ Unit			Product Number
MabSpeed™ rP202	10000	mL	5-105-04
	500	mL	5-105-02
	100	mL	5-105-01
	25	mL	5-105-00

*Please inquire us or below distributor for MabSpeed™ screening columns.

Related Data Sheets:
MabSpeed™ rP102 and rP111: No. 03-07-C-0101
Bioseparation Screening Columns : No. 03-01-C-0101

Available Documents:
Packing procedure for MabSpeed™ rP202
Use and care instructions for MabSpeed™ rP202
*Regulatory Support Files (RSF) are available upon request.

Notice

This information are given in good faith but without warranty, and this also applies where proprietary rights of third parties are involved. The application, use and processing of our products are beyond our control and therefore your own responsibility.

Distributor:

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