For ChromSpeed[™] and MabSpeeed[™],

Mitsubishi Chemical Corporation is pleased to offer pre-packed screening columns.

Introduction:

Commercial purifications in large scale begin at a small scale in methods development. In some cases, the developers and researchers wish to use media to pack their initial small columns. This case allows one to experience how the media can be handled physically and chemically, and one can utilize the knowledge later on during the scale-up periods. In other cases, the due to the speed and convenience of having a pre-packed column for media evaluation is necessary. These screening columns, available in sizes listed in Table 1, are suitable for evaluating different types of MabSpeed^{TM*}, shown in Table 2, and ChromSpeed™, shown in Table 3 for developing the purification conditions of biological target molecules such as proteins or nucleic acids. The screening columns are available in two column volumes of 1mL and

Highlights of screening columns:

- ➤ Packed with ChromSpeedTM for ion exchange media and with MabSpeed[™] for affinity chromatography media.
- ➤ Low cost, efficient, and alternative to self packing.
- Easy connections with AKTA®, FPLC®, and
- Offered in mixed or single chemistry packages of 4 or 5 columns

Screening:

A ChromSpeedTM screening column is available in two different particle sizes and four different functional groups. A MabSpeedTM screening column is available in two different particle sizes. Optimal selection of a particular media could involve screening of several media.



Table 1. Sizes and dimensions available for the screening columns.

	volume	Dimension	
ChromSpeed™	1 mL	7 mm ID × 26 mm	
MabSpeed [™]	5 mL	15.7 mm ID × 26 mm	

Table 2. Product line-up of MabSpeed[™] affinity chromatography media*

	Type of ligand	Particle diameter (μm)	DBC*** (g/L-media)
rP102		45	24
rP111	Protein-A	35	33
rP202**		45	TBA

MabSpeed™ *Screening columns of affinity chromatography media are available upon request.

**MabSpeedTM rP202 columns are available in Asia and will be available in other regions in the end of 2016.

***10% breakthrough, y-globulin, r.t. 3min

Table 3. Product line-up of ChromSpeed[™] ion exchange media

	Functional group	Particle diameter (µm)	Ion exchange capacity (eq/L-media)	Human γ- globulin SBC (g/L-media)
S103	-SO ₃ -	60	0.09	125
S101	-303	30	0.08	140
Q103	-N ⁺ (CH ₃) ₃	60	0.08	124
Q101	-N (CП3)3	30	0.08	130
CM103	-COO	60	0.11	114
CM101	-000	30	0.10	120
DA103	-N ⁺ H(CH ₃) ₂	60	0.13	81
DA101	DA101 -N H(CH ₃) ₂	30	0.11	98

ChromSpeed™ ion exchange media

separates molecules based on the ionic interaction of the molecules with the charged support. The net surface charge of proteins is dependent on the pH and ionic strength of the mobile phases. The development of optimal chromatography conditions requires knowledge of both the protein's pl and the pKa of the ion exchange media. In biopurifications purification biologics, ChromSpeed[™] can be used either in "bind/ elute mode" and/ or in "flow-through mode." Ion exchange media should be selected according to the properties of the feedstock and the objective of the process steps. ChromSpeedTM media designed for such a large molecules with large pore sizes, mainly dominated in the range of 500 to 1000 Å, offering large binding capacity. Some of typical applications is shown in Figure 1 and 2 as well as Figure 3.

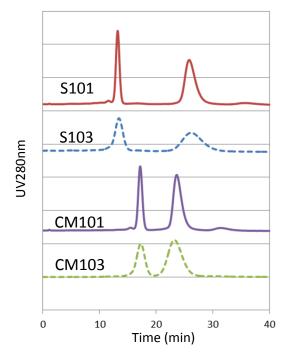


Figure 1. Standard protein screening with strong and weak cation media. Dotted lines represent media with larger particle size of $60 \mu m$. Data were taken with a conditions of: a column of 5 mm ID x 100 mm with eluent A of 20mM sodium phosphate (pH 6.5) and eluent B of A + 1.0M NaCl with a flow rate of 1.0mL/min (r.t. 2min) and a gradient of 0-100% B over $60 \mu m$. Sample was (a) cytochrome c (pl 9.3) + (b) lysozyme (pl 11.0) = $125 / 125 \mu m$ / $50 \mu m$.

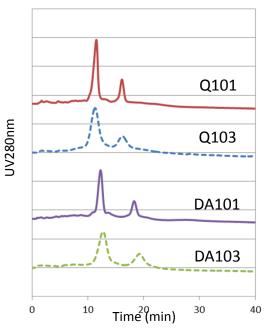


Figure 2. Standard protein screening with strong and weak anion media. Dotted lines represent media with larger particle size of 60 μ m. Experiments were conducted with conditions of a column of 5 mm ID x 100 mm with eluent A of 50mM Tris-HCl (pH 8.5) and eluent B of A + 1.0M NaCl with a flow rate of 1.0 mL/min (r.t. 2 min) and a gradient of 0-100% B over 6 0min. Sample was (a) Myoglobin (pl 7.5) + (b) Trypsin inhibitor (pl 4.5) = 250/50 ug / 25 uL.

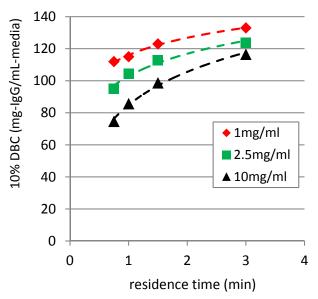


Figure 3. Effect of IgG concentration. Data was taken with a column of 5 mm ID x 100mm with buffer of 20mM Sodium Acetate (pH5.5). Flow rates were: 200, 400, 600, 800 cm/h (r.t. 3.0, 1.5, 1.0, 0.75 min). Samples were: a) 1.0, b) 2.5, c) 10 mg/mL in γ -globulin (IgG) concentrations.

Laboratory benchtop purifications:

MabSpeed™ rP111 was used for the purification of a monoclonal antibody from CHO cell culture supernatant with a concentration of 0.5 mg/mL at 3 minutes residence time in a 15 cm bed height column. Consecutively, the processed fluid was purified with ChromSpeedTM S101 and ChromSpeed[™] Q101, as a flow of Figure 4 shows, and the intermediate and final products were analyzed with regard to yield, dimers, aggregates, Protein A carry over, and Host Cell Proteins (HCP) as shown in the following Table 4.

Table 4. Representative data analyzed at each process described in Figure 4.

	Accumulated yield (%)	Dimers & aggregates (%)*	Protein-A (ppm)**	HCP (ppm)**
Starting Material	100	-	-	35717
rP111	98	<0.5%	4.4	3.1
S101	90	<0.5%	2.6	<0.5
Q101	88	<0.5%	2.4	<0.5

^{*} Data measured by size exclusion HPLC method.

Cell culture



Protein A affinity: MabSpeed™ rP111

Column: MabSpeed™ rP111 10 mm ID x 150 mm Sample: Clarified CHO cell culture, 0.5 mg/mL mab

(20.8 mg/mL-media)

Equilibration buffer: 20mM sodium phosphate, 150mM

NaCl, pH7.4

Elution buffer: 20mM Sodium Citrate, pH3.4

Flow: 300 cm/h, R.T. 3min



Cation exchange : ChromSpeed[™] S101

Column: ChromSpeed™ S101 5 mm ID x 200 mm Sample: MAb (51.3 mg/mL-media) after above Start buffer: 20 mM sodium acetate, pH 5.5

Elution buffer: 20 mM sodium acetate, 1M NaCl, pH 5.5

Flow: 300 cm/h, r.t. 4 min

Gradient: 5% (10 CV), 15% (20 CV), 100% (10 CV)



Anion exchange : ChromSpeed™ Q101

Column: ChromSpeed[™] Q101 5 mm ID x 50 mm Sample: mab (192 mg/mL-media), eluate after above

(pH adjusted to 6.0)

Start buffer: 20mM sodium acetate, pH 6.0

Flow: 300 cm/h, r.t. 1min

Figure 4. Process flow diagram of purification of a monoclonal antibody from CHO cell supernatant.



MabSpeedTM rP102 & rP111: No. 03-07-C-0101

MabSpeedTM rP202 : No. 03-07-C-0201

ChromSpeed[™] S101& S103 : No. 03-02-C-0101 ChromSpeed[™] Q101& S103 : No. 03-04-C-0101 ChromSpeed[™] CM101& CM103 : No. 03-03-C-0101 ChromSpeed[™] DA101& DA103 : No. 03-05-C-0101



^{**} HCP and Protein-A concentration were measured by typical ELIZA kit, available on market.

Ordering information:

Screening column: volume			Contents	Unit	Part
					Number
ChromSpeed [™] S103	1	mL	5 columns	set	7-103-01
ChromSpeed [™] Q103	1	mL	5 columns	set	7-103-00
ChromSpeed [™] CM103	1	mL	5 columns	set	7-103-03
ChromSpeed [™] DA103	1	mL	5 columns	set	7-103-02
ChromSpeed [™] 103Kit	1	mL	each (total of 4)	set	7-103-04
ChromSpeed [™] S103	5	mL	5 columns	set	7-104-01
ChromSpeed [™] Q103	5	mL	5 columns	set	7-104-00
ChromSpeed [™] CM103	5	mL	5 columns	set	7-104-03
ChromSpeed [™] DA103	5	mL	5 columns	set	7-104-02
ChromSpeed [™] 103Kit	5	mL	each (total of 4)	set	7-104-04
ChromSpeed [™] S101	1	mL	5 columns	set	7-101-01
ChromSpeed [™] Q101	1	mL	5 columns	set	7-101-00
ChromSpeed [™] CM101	1	mL	5 columns	set	7-101-03
ChromSpeed [™] DA101	1	mL	5 columns	set	7-101-02
ChromSpeed [™] 101Kit	1	mL	each (total of 4)	set	7-101-04
ChromSpeed [™] S101	5	mL	5 columns	set	7-102-01
ChromSpeed [™] Q101	5	mL	5 columns	set	7-102-00
ChromSpeed [™] CM101	5	mL	5 columns	set	7-102-03
ChromSpeed [™] DA101	5	mL	5 columns	set	7-102-02
ChromSpeed [™] 101Kit	5	mL	each (total of 4)	set	7-102-04

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:

^{*}Please inquire us or below distributor for MabSpeedTM screening columns.