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 ChromSpeed™ 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.
** HCP and Protein-A concentration were measured by typical ELIZA kit, available on market.

Related Data Sheets:
MabSpeed™ rP102 & rP111: No. 03-07-C-0101
MabSpeed™ 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

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: 20mM sodium acetate, 1M NaCl, pH5.5
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-resin), 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 culture supernatant.

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

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

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.

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THE KAITEKI COMPANY Mitsubishi Chemical Holdings Group
MITSUBISHI CHEMICAL CORPORATION

MabSpeed™
ChromSpeed™
XtalSpeed™

Bioseparation Screening Columns

Product Data Sheet
No.03-07-C-0101

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™*, 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 5mL.

Highlights of screening columns:

- Packed with ChromSpeed™ 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 HPLC
- Offered in mixed or single chemistry packages of 4 or 5 columns

Screening:

A ChromSpeed™ screening column is available in two different particle sizes and four different functional groups. A MabSpeed™ 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	Protein-A	45	24
rP111		35	33
rP202**		45	50

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

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

***10% breakthrough, γ-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		30	0.08	140
Q103	$-N^+(CH_3)_3$	60	0.08	124
Q101		30	0.08	130
CM103	$-COO^-$	60	0.11	114
CM101		30	0.10	120
DA103	$-N^+H(CH_3)_2$	60	0.13	81
DA101		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 pI and the pKa of the ion exchange media. In biopurification or purifications of 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. ChromSpeed™ media are 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.

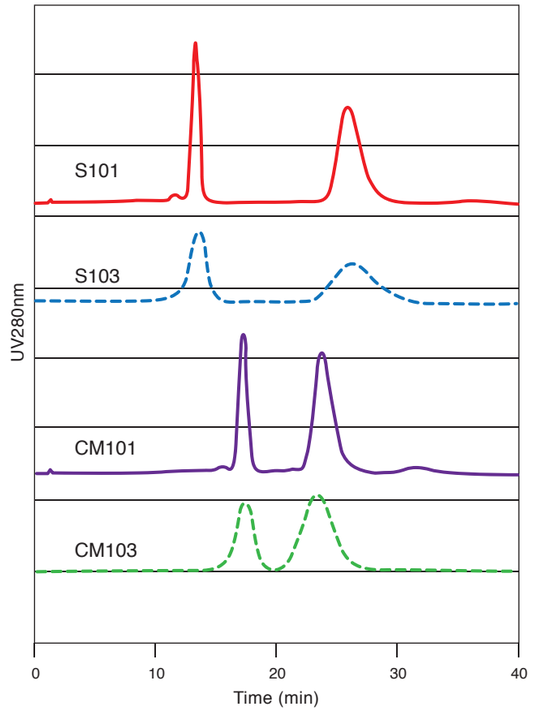

Figure 1. Standard protein screening with strong and weak cation media. Dotted lines represent media with larger particle size of 60 µm. Data were taken with a conditions of: a column of 5 mm ID x 100 mm with eluent A of 20mM sodium phosphate (pH 6.5) and eluent B of A + 1.0M NaCl with a flow rate of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

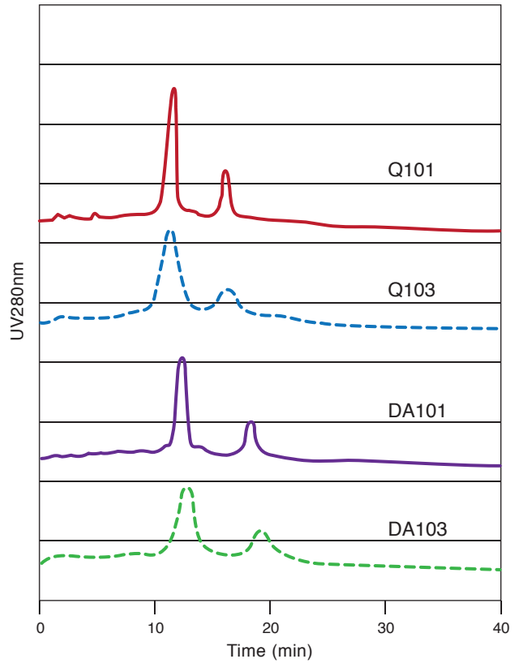

Figure 2. Standard protein screening with strong and weak anion media. Dotted lines represent media with larger particle size of 60 µ m. Experiments were conducted with conditions of a column of 5 mm ID x 100 mm with eluent A of 50mM Tris-HCl (pH 8.5) and eluent B of A + 1.0M NaCl with a flow rate of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

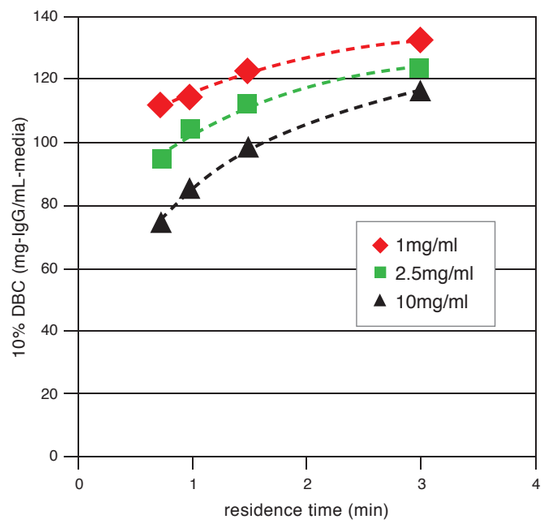

Figure 3. Effect of IgG concentration. Data was taken with a column of 5 mm ID x 100mm with buffer of 20mM Sodium Acetate (pH5.5). Flow rates were: 200, 400, 600, 800 cm/h (r.t. 3.0, 1.5, 1.0, 0.75 min). Samples were: a) 1.0, b) 2.5, c) 10 mg/mL in γ-globulin (IgG) concentrations.