SARTORIUS

Product Datasheet

RoboColumn®* Formats for HTPD

Prepacked Columns for Resin Screening and Method Development

Product Information

RoboColumn® are miniaturized chromatography columns pre-packed with chromatography resins. They are designed for fully automated and parallel chromatographic separations with robotic liquid handling workstations, such as the Freedom EVO® from Tecan.

RoboColumn® are packed with ion exchange, mixed-mode, affinity or any other chromatography resins upon request. The column units are packed by Repligen and are identical to OPUS RoboColumn®. Two formats are available, 200 µL and 600 µL, with a bed height of 10 and 30 mm respectively.

Features and Benefits

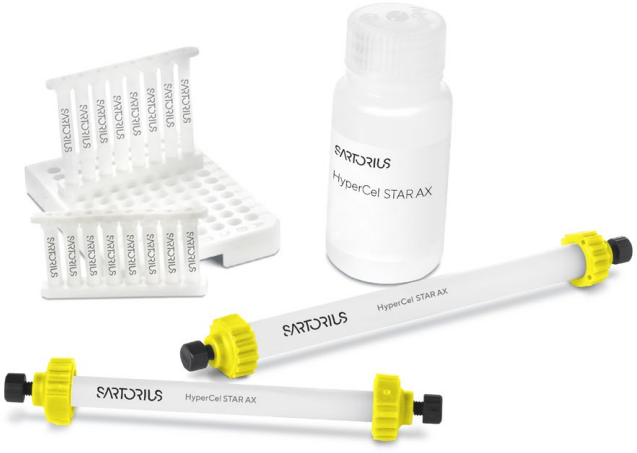
- Column format and high quality packing enables mimicking of all steps of a chromatography separation in dynamic mode. Data obtained are comparable to those obtained in larger columns
- Miniaturized format facilitates screening of chromatography resins, reduces sample consumption, and saves time
- Perfectly fitted to Design of Experiment (DoE) requirements, allowing a large amount of experimental work in a minimum of time
- 96-well format allows easy integration with automation systems for High Throughput Process Development (HTPD). Multiple parameters can be tested in parallel, e.g., residence time

*RoboColumn is a registered trademark of Repligen GmbH

Introduction

The 200 µL column volume is recommended for resin and process conditions screening, while the 600 µL is used for purification process optimization or when a residence time above 4 minutes is required (e.g., capacity study on mixed-mode resins).

The columns are supplied as rows of eight pre-packed units, with two removable silicon cover seals for proper storage. A 96-well array plate is available to arrange up to 96 RoboColumn® units, and the user makes the column selection as needed. The miniturized columns can be reused.



Technical Specifications

RoboColumn®		
Column volume	200 µL	600 µL
Bed height	10 mm	30 mm
Column inner diameter	5 mm	
Column material	Polypropylene	
Chemical stability	All commonly used aqueous buffers, pH 1 to 14	
Avoid	Halogenated organic solvents, hexane	
Storage solution	20% ethanol | 1 M NaCl	
Recommended storage temperature	2 to 8 °C	
Working temperature	4 to 30 °C	
Maximum working pressure	Up to 8.0 bar g (116 psi g)	
Flow rate	16 to 1,000 cm/hr[1]	
Laser label	Placed on every individual column body, and containing: Product number Resin name Column volume in µL	

96-Well Array Plate	
Size	128.3 × 86.0 × 14.0 mm
Material	Polyoxymethylene copolymer
Number of wells	96

[1] The Liquid Transfer menu of the Te-Chrom™* Wizard enables set up of the flow rate for each individual dispensing and chromatography step (5.1.1.6 Liquid Transfer, Te-Chrom™ Wizard Software Manual, 396076, en, V1.0).

*Te-Chrom is a trademark of Tecan Trading, Ltd.

Applications

RoboColumn® are suitable for a large variety of applications such as:

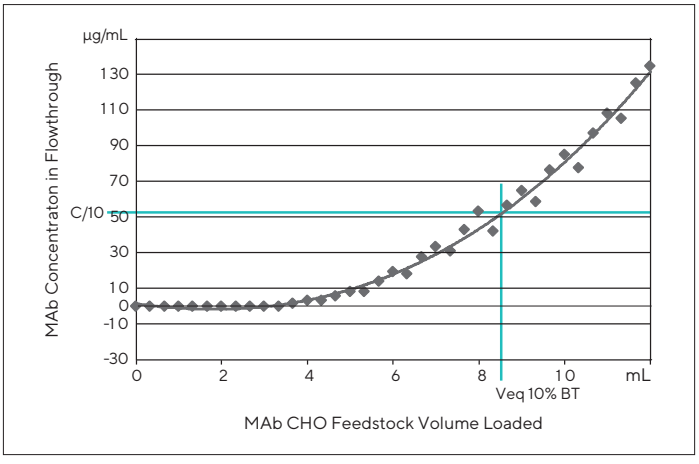
- Parallel screening of chromatography resins
- Parallel screening and optimization of chromatographic conditions in downstream process development (conductivity, pH)
- Scale down experimental work
- Determination of dynamic binding capacity
- Determination of the optimal residence time
- Optimization of the regeneration step

Application Example: Development of the Capture Step of a Monoclonal Antibody (MAb) Expressed in a Crude CHO Feedstock

MAb binding conditions were tested on 3 mixed-mode resins and dynamic binding capacity (DBC) was evaluated based on breakthrough (BT) curve (Figure 1).

The RoboColumn® was overloaded with MAb CHO feedstock at 0.52 mg/mL and flowthrough (FT) was collected as one-column-volume (CV) fractions (200 µL each) in 350 µL 96-well plate. The amount of MAb recovered in the FT fractions was evaluated using Bio-Layer Interferometry (BLI) with protein A sensor (Octet® System, ForteBio). It was plotted against the volume of feedstock loaded per RoboColumn® unit. 1/10 of the MAb concentration (C/10) was 0.052 mg/mL (52 µg/mL in Figure 1). The volume equivalent at 10% BT (Veq10%BT) was the volume of MAb loaded when reaching C/10 in FT fractions (8.56 mL in Figure 1).

Figure 1: *Breakthrough Curve on MEP HyperCel Resin in 200 µL RoboColumn®*



Breakhrough during loading of MAb CHO feedstock (0.52 mg/mL) at ~4 minute RT.

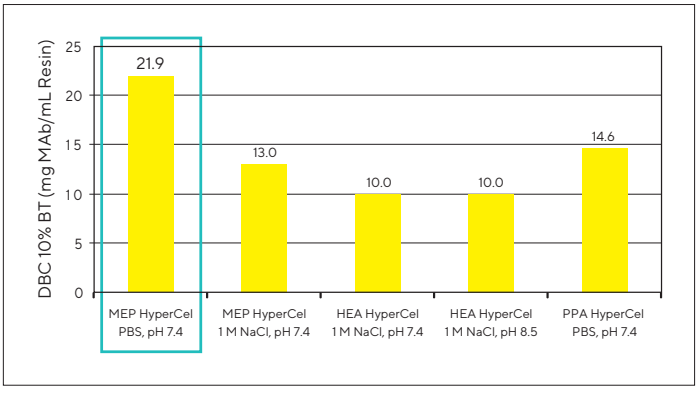
DBC10% BT (mg/mL resin) =

$$\frac{\text{Veq10\%BT (mL)}^{*} \times \text{MAb concentration (mg/mL)}}{\text{Column volume (mL)}}$$

* void volume was taken into account

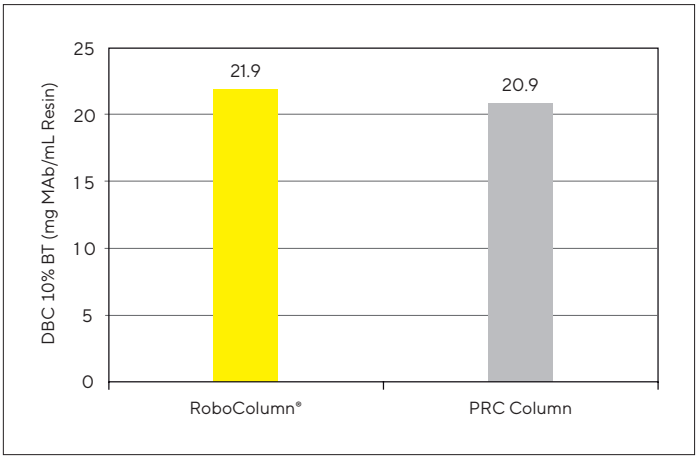
Using this breakthrough curve method, the DBC for MEP, HEA and PPA HyperCel resins was determined on 200 µL RoboColumn® (Figure 2).

Figure 2: *DBC on 3 Mixed-Mode HyperCel Resins in RoboColumn® for Different Binding Conditions*



This comparison showed that MAb binding was maximized on MEP HyperCel resin in PBS, pH 7.4. The selected condition was then transferred to 1 mL PRC column for confirmation of the DBC, approximately 21 mg/mL (Figure 3).

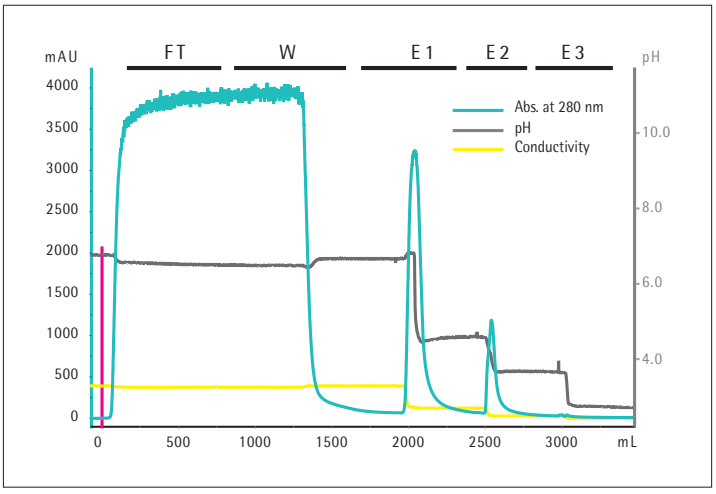
Figure 3: *DBC on MEP HyperCel Resin in RoboColumn® and in PRC Column*



A 335-fold scale-up of the MAb capture step was finally carried out from 200 µL RoboColumn® to a lab-scale column (2.5 cm I.D) packed with 67 mL MEP HyperCel resin (Figure 4).

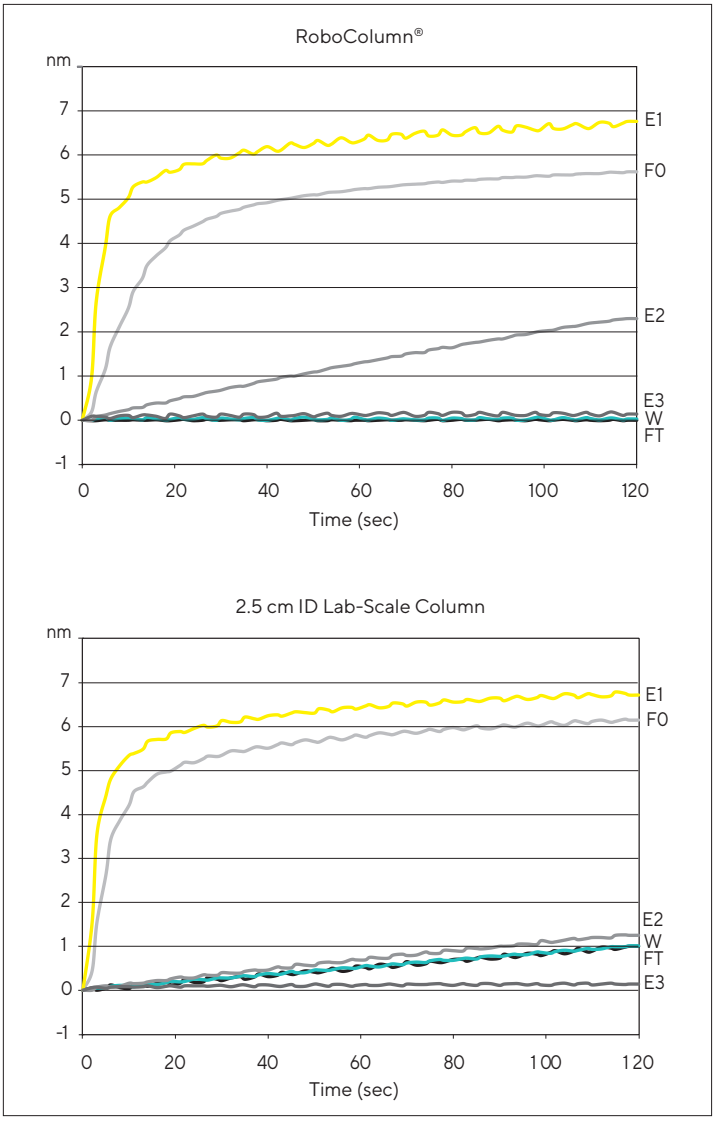
The analysis of fractions obtained on RoboColumn® and a 2.5 cm ID lab-scale column were in the same magnitude. Comparable data in terms of recovery (100%) and purity (90%) were evidenced (Figure 5, Table 2).

Figure 4: *335-fold Scale-Up of the MAb Capture Step from RoboColumn® to a 2.5 cm ID Lab-Scale Column*



Chromatogram of MAb Capture Step on a 2.5 cm ID lab-scale column (67 mL). Equilibration in PBS, pH 7.4. Loading of MAb feedstock = 21 mg MAb/mL MEP HyperCel resin. Residence time = 4 minutes; FT: Flowthrough; W: Wash; E1, E2 and E3: Elution at pH 5, 4 and 3 respectively.

Figure 5: *Fraction Analysis From RoboColumn® and a 2.5 cm ID Lab-Scale Column Using BLI*



Interaction of the MAb with protein A sensor was measured using BLI. Signal is correlated to MAb concentration using a standard curve (data not shown). Residence time = 4 minutes. F0: Sample load; FT: Flowthrough; W: Wash; E1, E2 and E3: Elution at pH 5, 4 and 3 respectively.

Table 2: *Summary of the MAb Capture Step on RoboColumn® or a 2.5 cm ID Lab-Scale Column*

Column Type	Volume [mL]	MAb Loaded [mg]*	Loading [mg MAb/mL resin]	Recovery [%]*	Purity [%]**
RoboColumn®	0.2	4	22	102	86
Lab scale column 2.5 cm I.D.	66.7	1376	21	99	91

* Evaluated using BLI technology (Octet System, ForteBio)
** Evaluated using LabChip* GXII technology (PerkinElmer)

Handling

RoboColumn® are designed for robotic handling.
When using the high-throughput process development approach for RoboColumn®, it is suggested to use Design of Experiments (DoE) criteria for the experimental set-up. This enables screening of many different chromatographic parameters such as resin type, pH, conductivity | ionic strength, etc., efficiently and simultaneously.

For experimental set-up and the configuration of the 96-well array plate, the Te-Chrom™ Wizard is recommended. The Te-Chrom™ Wizard is a dialog-based graphic user interface offered by Tecan and based on the Freedom EVOware software. The Te-Chrom™ Wizard was developed for the use of RoboColumn® and provides the possibility to configure hardware, plate layout, and the chromatographic process as well as to set all process relevant chromatographic parameters, e.g., flow rate, volume without direct script writing.

Ordering Information

Description	Part Number
RoboColumn® MEP HyperCel 200 µL, row of 8	SR2MEP
RoboColumn® MEP HyperCel 600 µL, row of 8	SR6MEP
RoboColumn® HEA HyperCel 200 µL, row of 8	SR2HEA
RoboColumn® HEA HyperCel 600 µL, row of 8	SR6HEA
RoboColumn® PPA HyperCel 200 µL, row of 8	SR2PPA
RoboColumn® PPA HyperCel 600 µL, row of 8	SR6PPA
RoboColumn® HyperCel STAR AX 200 µL, row of 8	SR2STARAX
RoboColumn® HyperCel STAR AX 600 µL, row of 8	SR6STARAX
RoboColumn® CM Ceramic HyperD F 200 µL, row of 8	SR2CMCHDF
RoboColumn® CM Ceramic HyperD F 600 µL, row of 8	SR6CMCHDF
RoboColumn® CMM HyperCel 200 µL, row of 8	SR2CMM
RoboColumn® CMM HyperCel 600 µL, row of 8	SR6CMM
96-well RoboColumn array plate	SR96WAP

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