

OPUS[®] RoboColumn[®] Pre-packed Chromatography Columns

USER GUIDE



01-08R-E02

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1 Introduction

OPUS® RoboColumns® are small chromatography columns for fully automated and parallel chromatographic separations with robotic liquid handling workstation such as Tecan's Freedom EVO®. RoboColumns® are available pre-packed with any one from a large selection of commercially available chromatographic media of different functionalities including AIEC, CIEC, HIC, SEC, AC, HCIC and MMC mode of operation. Bed containment between two filter frits and professional packing with consideration to individual material compression requirements ensure high efficiency and peak symmetry similar to that of preparative and process separation columns and distinguishes the system from the current filter based systems for simple on/off sample equilibration operation. Up to 96 RoboColumns® can be individually and easily arranged / fixed onto a 96 well array plate according to application requirements. As a result of this modular design, RoboColumn® technology is used in a broad spectrum of applications, such as parallel screening and optimization of chromatographic conditions, scale-down experiments, sample preparation prior to subsequent analysis like MS, ELISA or CGE/SDS-Page as well as for the concentration of low titer compounds. All chromatography media used in RoboColumns® are also available in larger pre-packed OPUS® MiniChrom columns of different geometries from 0.2 – 10.0 mL for the performance of scale-up studies by using common one-channel stand-alone LC-systems.

2 Characteristics

RoboColumns® are ready-to-use 96-well formatted chromatography columns pre-packed with almost any commercial chromatography resins. The available chromatography resins are listed in Repligen's interactive online RoboColumn® configurator.

www.repligen.com/resources/configurators/robocolumn/

RoboColumns® are available in a volume range from 50 µL to 600 µL.

Table 1. presents available column geometries and characteristics of RoboColumns®.

Table 1. OPUS® RoboColumn® characteristics

Plate size	128.3 x 86.0 x 14.0 mm					
Plate material	Polyoxymethylene copolymer					
Number of wells	96					
Column volume	50 µL	100 µL	200 µL	450 µL	500 µL	600 µL
Bed height	2.5 mm	5.0 mm	10.0 mm	22.5 mm	25.0 mm	30.0 mm
Column inner diameter	5 mm					
Column material	Polypropylene					
Chemical stability	All commonly used aqueous buffers, pH 1 – 14, organic solvents ¹					
Avoid	Halogenated organic solvents, hexane					
Storage solution	<ul style="list-style-type: none"> • AIEC and CIEC: 20 % ethanol + 150 mM sodium chloride • HIC, SEC, AC and MMC: 20 % ethanol • CHT, CFT (Bio-Rad): 20 % ethanol + 10 mM sodium phosphate, pH 6.8 • HyperCel™ (PALL): 30 % isopropanol + 0.1 M sodium phosphate, pH 4.3 					

	<ul style="list-style-type: none"> • ProSep® (Millipore): 1 % benzyl alcohol + 0.1 M sodium acetate, pH 5.1
Recommended storage temperature	<ul style="list-style-type: none"> • AIEX, CIEX, HIC, SEC and MMC: +4°C to +30°C • AC: +4°C to +8°C
Working temperature	+4°C to +30°C
Max. working pressure	Up to 8.0 bar
Flow velocity	16 – 1000 cm/h ²
Laser label	Placed on every individual column body and containing: <ul style="list-style-type: none"> • Product number • Resin name • Column volume in µL

¹ **Note:** The chemical stability of the packed chromatography media has also to be taken into consideration. For more information please refer to the manufacture’s resin specification.

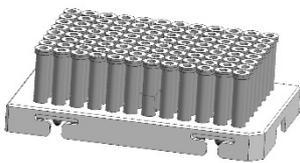
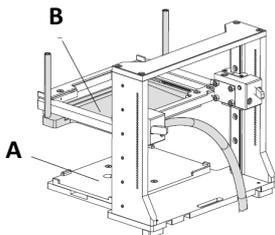
² The Liquid Transfer menu of the Te-Chrom™ Wizard enables to set up the flow rate for each individual dispensing and chromatography step (5.1.1.6 Liquid Transfer, Te-Chrom™ Wizard Software Manual, 396076, en, V1.0).

3 Advice on handling

3.1 Equipment

RoboColumns® are designed for robotic handling. Table 2 is a guide for the equipment required for robotic handling of RoboColumns® using Tecan’s Freedom EVO® workstation.

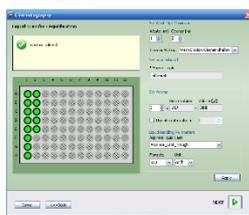
Table 2. Recommended equipment for automated handling of RoboColumns®

Equipment	Details	Tips and tricks
RoboColumn® 96-well base plate 	Arrange up to 96 individual RoboColumns® onto the 96 well array plate according to application requirements.	Do not mix short (50, 100, 200 µL) and long (450, 500, 600 µL) RoboColumns®. Make sure that RoboColumns® are well clicked into the 96 well array plate before using them.
Te-Chrom™ Module (A) 	Use the Te-Chrom™ module to integrate the RoboColumn® array onto the robotic worktable. <ul style="list-style-type: none"> • Height adjustable • Waste option (B) 	Use the Te-Chrom™ module in combination with a plate stacker Te-Stack™ for the optimized handling and storage of collection plates. Use the waste container to drain off e.g. conditioning, regeneration solvents. Make sure that the RoboColumn® array is in an appropriate distance to the collection plate before starting the experiment.

Te-Chrom™ Shuttle

Use the Te-Chrom™ Shuttle to move a collection plate under the RoboColumn® array to fractionate the flow-through coming from the columns' outlets.

Use the RoMa arm to transport the collection plate from a pre-defined transfer position to any destination on the robotic worktable e.g. plate reader, hotels, auto sampler, etc. for further analysis e.g. UV measurement, ELISA, HPLC, MS, etc.

Te-Chrom™ Wizard

Use the Te-Chrom™ Wizard to configure the chromatographic process and to set all process relevant parameters e.g. volume, flow rate, etc.

Assign labels e.g. resin name and column types by using the plate layout configurator. Use the neutralization option to adjust the pH of the fractions after the acidic elution of mAbs. **Note:** Confirm with Tecan suitability for every specific application.

Plate reader

Use the plate reader for data evaluation e.g. UV measurement and determination of protein concentration.

Calculate the precise volume of your collection fractions for subsequent data plotting.

Reagent and sample reservoirs

A broad spectrum of troughs, tubes and plates can be positioned onto the robotic worktable by using the appropriate Tecan carrier.

Use an 8-, 48- or 96- deep well reservoir for buffer solution/ preparation.

Collection plate

Use any type of multi-well plate which matches the ANSI/SDS standard (UV- or non- UV readable).

Use a half area 96 well microplate to collect fractions in a volume range from 25 µL to 175 µL. Use a standard 96 well microplate to collect fractions in a volume range from 75 µL to 340 µL. Use 96 deep well reservoirs to collect high volume fraction up to 2.0 mL.

3.2 Sample Preparation

We recommend loading a clarified sample on RoboColumns®, since unclarified sample may cause clogging of the top and bottom filter frits, which impacts the column life time.

3.3 Experimental Setup

RoboColumns® are suitable for a large variety of application such as parallel screening and optimization of chromatographic conditions in downstream process development, sample preparation prior to subsequent analysis like MS, ELISA or CGE/SDS-Page as well as the concentration of low titer compounds in drug discovery.

The modular design allows arranging up to 96 individual RoboColumns® on the corresponding 96 well array plate according to applications requirements.

The unique column design and the continuous mode of chromatographic operation enable to perform real chromatographic separations and to determine dynamic chromatographic parameters e.g. dynamic binding capacity (DBC) and retention time (R_t).

When using the high-throughput process development approach for RoboColumns®, it is suggested to use Design of Experiments (DoE) criteria for the experimental set-up which enables to screen many different chromatographic parameters such as resin type, pH, conductivity / ionic strength, salt type, etc. efficiently and simultaneously.

For experimental set-up and the configuration of the RoboColumn® array plate the Te-Chrom™ Wizard is recommended, see [Section 3.4](#).

Examples of experimental set-ups are described in RoboColumn® application notes; see RoboColumn® literature, [Section 6.3](#).

3.4 Te-Chrom™ Wizard

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 RoboColumns® 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, see Fig 1.

Please confirm with Tecan suitability for every specific application.

For more detailed information please refer to the Te-Chrom™ software manual.

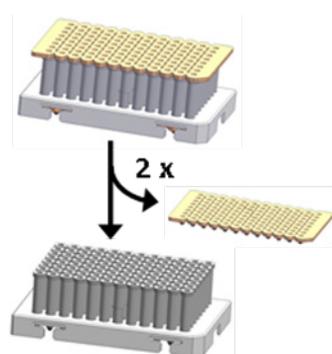
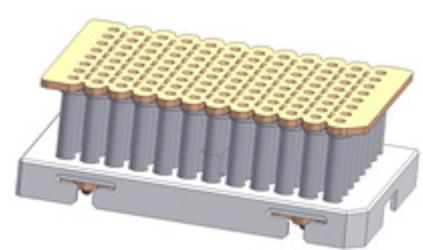
Figure 1. Te Chrom™ wizard features



4 Protocol

The protocol is designed as a general guideline for working with RoboColumns®. Adjustment may be required depending on type of application, sample, resin type and RoboColumn® dimension. One product package contains 8 to 96 individual pre-packed RoboColumns® arranged on a 96 well array plate and two removable cover seals for the appropriate storage of RoboColumns®, see Fig. 2.

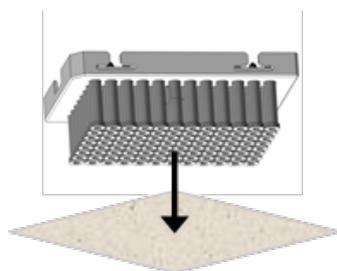
Figure 2. 96 RoboColumn® array



4.1 Detailed protocol

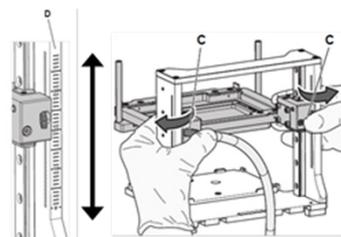
1. Remove cover seals

- A. Gently peel off at first the top seal from the RoboColumn® array.
- B. Gently peel off the bottom seal from the RoboColumn® array.



1. Remove excess storage solution from needle adaptor inlet

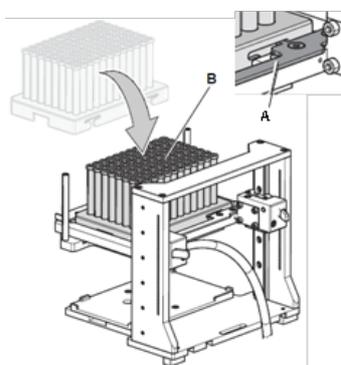
Remove remaining storage solution in the top part of the columns by blotting the RoboColumn® array upside down on a soft paper tissue.



2. Te-Chrom™ - Adjusting the operating height

Press and hold the fastener (C) to set the Te-Chrom™ to the required height of the Te-Chrom™ module scale (D).

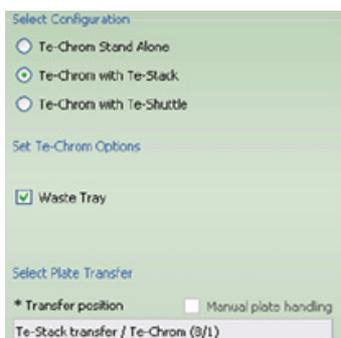
Note: Make sure that the RoboColumn® array is in a position as close as possible to the collection plate before starting the experiment.



3. Te-Chrom™ - Loading RoboColumns®

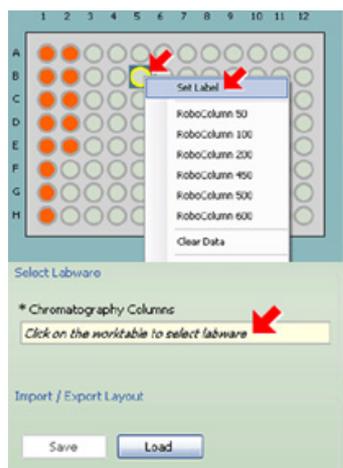
- A. Move the locking slider (A) to the left mechanical stop.
- B. Load the RoboColumn® array (B).
- C. Move the locking slider (A) to the right mechanical stop to lock the RoboColumn® array.

Note: Make sure that all RoboColumns® are well fixed into the 96 well array plate before starting the experiment.



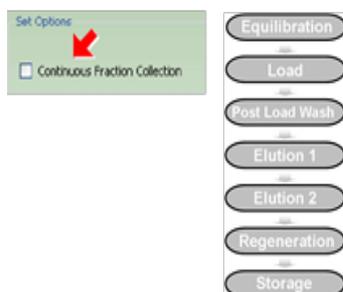
4. Te-Chrom™ Wizard – Experimental setup

- A. **Hardware configuration**
 - Select the appropriate configuration of the Te-Chrom™
 - Check if your Te-Chrom™ is equipped with a waste tray.
 - Define a transfer position for the collection plate.



B. Plate layout

- Select the RoboColumn® labware.
- Define the required RoboColumn® matrix.
- Select the RoboColumn® dimension.
- Set a desired label e.g. resin name.
- Import or export the plate layout.



C. Process configuration

- Define the chromatographic workflow by setting a desired number of process steps.
- Use the Continuous Fraction Collection option to collect fractions over multiple plates, without leaving gaps between plates pipetted in different process steps and to optimize the consumption of collection plates.



D. Configure your process steps

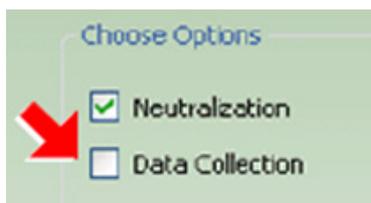
- Edit a process step name e.g. Equilibration
- Check *the Process Full Plate option* accordingly:
 - When checked: The full RoboColumn® array will be processed.
 - When not checked: The RoboColumn® array is processed column per column.
- Set parameters for tip washing.
- Set individual chromatographic parameters.
- Select the source of liquid.
 - Set the dispensing volume.
 - Set the required liquid class for aspiration.
 - Set the required flow-rate [cm/h].



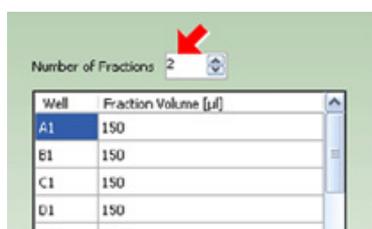
Note: Calculate the required residence time (\square) by dividing the RoboColumn® volume (CV) by the volumetric flow-rate (uv). $\square = CV/uv$



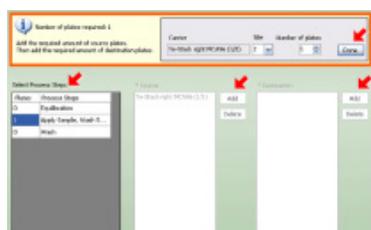
- Check the waste option accordingly:
 - When checked: The waste tray will be engaged by the RoMa / LiHa.
 - When not checked: The flow-through will be collected in a desired collection plate.



- Check the *Neutralization* option to perform a pH adjustment after e.g. acidic elution of mAbs.
- Check the *Data Collection* option to evaluate the collection plate in a required reader device by e.g. UV measurement.

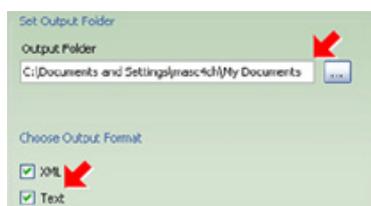


- Select the number of fractions according to the given dispensing volume for the chromatography step if the waste option is not checked.



E. Plate handling

- Add the required amount of source plates.
- Add the required amount of destination plates.



F. Reporting

- Set output folder.
- Select output format.

After the experimental set-up the Te Chrom™ wizard will generate a chromatographic method in Freedom EVOware® software automatically.



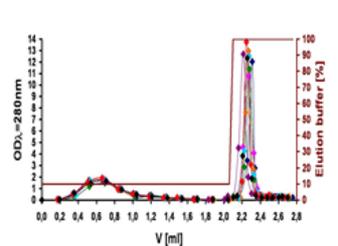
6. Run chromatographic method

Note: Make sure that all required solvents and consumables e.g. collection plates are in the designated position on the robotic worktable, before running the chromatographic method.

7. Data evaluation

- Determine the precise volume of the collected fractions by using an appropriate plate reader.
- Determine the protein concentration by using UV measurement.

Note: Transfer the collection plate to any location on the robotic worktable or to secondary devices e.g. auto sampler for further analysis e.g. HPLC, MS, ELISA, CGE/SDS-Page after path length detection by using the RoMa.



8. Data plotting

- Plot the UV data against the fraction volume to create a pseudo-chromatogram.

Note: Make sure that blank values of the used solvents are subtracted from the UV data before plotting.

9. Regeneration and storage

- For appropriate regeneration of RoboColumns® please refer to manufacture’s recommendation for the corresponding resin.
- For appropriate storage of RoboColumns® please refer to Table 1 RoboColumn® characteristics.

5. Troubleshooting guide

Fault	Possible cause	Action
RoboColumns® are clogged.	<ul style="list-style-type: none"> • The sample is too viscous. • There is too much cell debris in the sample. • The column has been regenerated and used too many cycles. 	<ul style="list-style-type: none"> • Increase dilution of the cell paste before lysis, or dilute after the lysis. • Centrifuge and/or filtrate the sample if unclarified sample has been used. • Reduce the number of cycles.
RoboColumns® are leaking.	<ul style="list-style-type: none"> • Columns are clogged. • The o-ring sealing is damaged. 	<ul style="list-style-type: none"> • See above. • Check the taught X- and Y coordinates for RoboColumn® labware for precision. • Check the insertion depth (16 mm from

	<ul style="list-style-type: none"> The insertion depth of the robotic needle is incorrect. 	the top of the column) of the robotic needle.
RoboColumns® are lifted up from the 96 well array plate during the pull out of the robotic needles.	<ul style="list-style-type: none"> RoboColumns® are not well fixed into the 96 well array plate. 	<ul style="list-style-type: none"> Make sure that all RoboColumns® are well fixed into the 96 well array plate before starting the experiment.
Low protein recovery in the elution fraction(s).	<ul style="list-style-type: none"> The residence time for sample loading is too low. 	<ul style="list-style-type: none"> Decrease the flow-rate for sample loading.

6. Ordering information

For information about related products, accessories and related literature, see online information at: www.repligen.com/products/downstream-solutions/opus/process-development/.

6.1 RoboColumns®

Product	No. supplied	Part #
96 well RoboColumn® array plate	1	00000507
Cover seals, top, bottom	1	00000008
Application support	on request	00000297
RoboColumn®, 50 µL	8-row	XXXXXX08R-02
RoboColumn®, 100 µL	8-row	XXXXXX08R-01
RoboColumn®, 200 µL	8-row	XXXXXX08R
RoboColumn®, 450 µL	8-row	XXXXXX08R-22.5
RoboColumn®, 500 µL	8-row	XXXXXX08R-25
RoboColumn®, 600 µL	8-row	XXXXXX08R-30

The available chromatography media are listed in Repligen's interactive online RoboColumn® configurator.

www.repligen.com/resources/configurators/robocolumn/

6.2 Related products

Product	No. supplied	Part #
96 well MiniColumn array plate	1	00000004
CentriColumn, 50 µL	8-row	XXXXXX08
CentriColumn, 100 µL	8-row	XXXXXX08-01
CentriColumn, 200 µL	8-row	XXXXXX08-02
PipetColumn, 50 µL	8-row	XXXXXX08P-02
PipetColumn, 100 µL	8-row	XXXXXX08P-01
PipetColumn, 200 µL	8-row	XXXXXX08P
PipetColumn, 450 µL	8-row	XXXXXX08P-22.5

PipetColumn, 500 µL	8-row	XXXXXX08P-25
PipetColumn, 600 µL	8-row	XXXXXX08P-30
PipetColumn BasicKit	1	00000159
PipetColumn Q - Starter Kit	1	00000104
PipetColumn S - Starter Kit	1	00000105
PipetColumn HIC - Starter Kit	1	00000106
PipetColumn ProteinA - Starter Kit	1	00000107
PipetColumn HA - Starter Kit	1	00000108
PipetColumn IMAC - Starter Kit	1	00000109
PipetColumn Individual - Starter Kit	1	00000055

6.3 Related literature

RoboColumn® literature	Part #
Poster: Small scale automated high throughput chromatographic separations in process development, in-process monitoring and validation of biopharmaceutical production	04-08R-E01 TS
Poster: Automated parallel chromatographic separations in process development	05-08R-E01 TS
Poster: Innovative high throughput protein purification in 96-array format	06-08R-E01 TS
Tecan: Te-Chrom™ operational manual	Contact Tecan
Tecan: Te-Chrom™ software manual	Contact Tecan

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