

Technical

User Guide

RoboColumns

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INTRODUCTION

RoboColumns are miniaturised chromatography columns, available pre-packed with Astrea Bioseparations' range of chromatography adsorbents and resins.

Suitable for use in a range of applications including high-throughput screening of chromatography media, optimization of process conditions, and scale-down experimental work, RoboColumns are supplied as a row of eight pre-packed columns in volumes of 200 µL. Multiple rows can be arranged onto a 96-well array plate for parallel experiments.

All chromatography media used in the RoboColumns is available in larger pre-packed columns and in bulk for use at scale.

RoboColumns are designed for use with automated robotic liquid handling systems, such as the Freedom EVO® from Tecan.

Properties of RoboColumns

Technical characteristics of RoboColumns

COLUMN VOLUME:	200 µL
BED HEIGHT:	10 mm
COLUMN INNER DIAMETER:	5 mm
COLUMN MATERIAL:	Polypropylene
CHEMICAL STABILITY:	All commonly used aqueous buffers and co-solvents, pH 1 - 14 ¹
AVOID:	Halogenated organic solvents, hexane
STORAGE SOLUTION:	IEX: 20% Ethanol, 150 mM NaCl HIC: 20% EtOH
RECOMMENDED STORAGE TEMPERATURE:	2 - 30 °C
MAXIMUM WORKING PRESSURE:	Up to 8 bar (116 psi g)
FLOW RATE CAPABILITIES:	16 - 1000 cm/h ²

¹ The pH stability of the packed chromatography media should be adhered to for operation. Refer to the respective Technical User Guide for this information.

² The maximum operational flow rate of the packed chromatography media should be adhered to for operation. Refer to the respective Technical User Guide for this information.

Operating Instructions

RoboColumns are intended for use with robotic liquid handling systems only. The below information outlines equipment needed for using RoboColumns with the Tecan Freedom EVO® workstation.

Recommended equipment for robotic handling of RoboColumns

Equipment	Details	Advice
96-well base plate	Arrange up to 96 individual RoboColumns onto the 96-well array plate according to application requirements.	<ul style="list-style-type: none">Do not mix short (50, 100, 200 µL) and long (450, 500, 600 µL) RoboColumns.Ensure each RoboColumn is securely in place in the 96-well array plate prior to use.
Te-Chrom™ module ¹	Use the Te-Chrom™ module to integrate the RoboColumn array plate onto the height adjustable robotic worktable.	<ul style="list-style-type: none">Use the Te-Chrom™ module in combination with a plate stacker (Te-Stack™) for optimized handling and storage of collection plates.Use the waste container to drain off solvents not required for analysis, e.g., conditioning, regeneration solvents.Ensure the RoboColumn array is in an appropriate location relative 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 plate to fractionate the flow-through coming from each column's outlet.	<ul style="list-style-type: none">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 ²	Use the Te-Chrom™ Wizard to configure the chromatographic process and to set all process relevant parameters, e.g., volume, flow rate, etc.	<ul style="list-style-type: none">Use the plate layout configurator to assign labels such as resin name and column type.Use the neutralization option to adjust the pH of the fractions after acidic mAb elution.Consult with Tecan for application-specific needs.
Plate reader	Use the plate reader for data evaluation, e.g., UV measurement and determination of protein concentration.	<ul style="list-style-type: none">Calculate the precise volume of your collection fractions for subsequent data plotting.
Reagent and sample reservoirs	A wide variety of troughs, tubes, and plates can be positioned onto the robotic worktable using the appropriate Tecan carrier.	<ul style="list-style-type: none">Use an 8-, 48- or 96-deep well reservoir to prepare buffer solution/s.

Collection plate	Use any type of multi-well plate which matches the ANSI/SDS standard (UV- or non-UV readable).	<ul style="list-style-type: none"> • Use a half area 96-well microplate to collect fractions with a volume of 25 μL to 175 μL. • Use a standard 96-well microplate to collect fractions with a volume of 75 μL to 340 μL. • Use 96-deep well reservoirs to collect high volume fractions up to 2.0 mL. 1x 5mL Octyl PuraBead[®] HF
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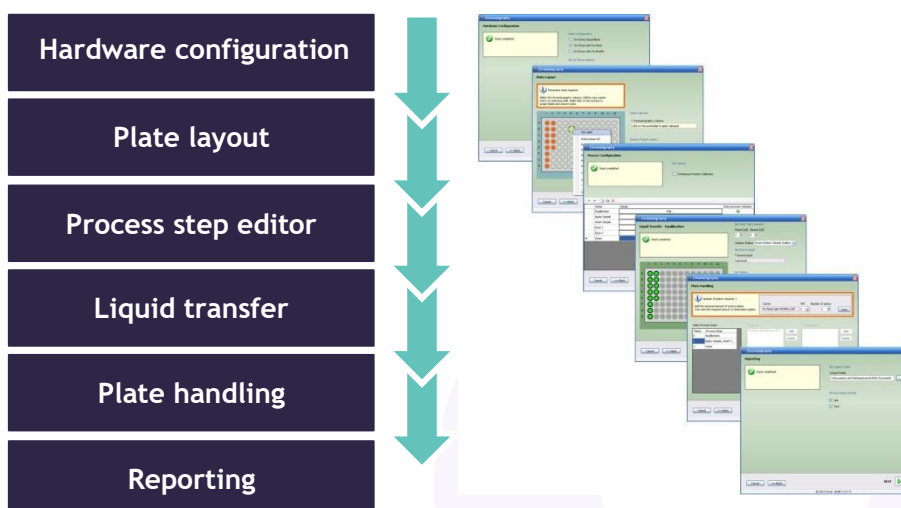
¹ For more detailed information about the Te-Chrom Module, see the Te-Chrom Operating Manual from Tecan.

² For more detailed information about the Te-Chrom Wizard, see the Te-Chrom Wizard Software Manual from Tecan.

It is recommended that samples are clarified before application to the RoboColumns to prevent impairment of use by blocking the frits.

When utilizing RoboColumns for high throughput screening and process development, using Design of Experiment (DoE) for the experimental set-up is recommended. This allows for multiple conditions and parameters to be evaluated simultaneously, including pH and salt concentrations.

For experimental set-up and the configuration of the 96-well array plate with the Freedom Evo[®], the Te-Chrom[™] Wizard is recommended. The Te-Chrom[™] Wizard is a dialog-based graphic user interface offered by Tecan developed for the use of RoboColumns. The software provides the possibility to configure hardware, plate layout, and the chromatographic process as well as to set all process relevant chromatographic parameters without direct script writing.

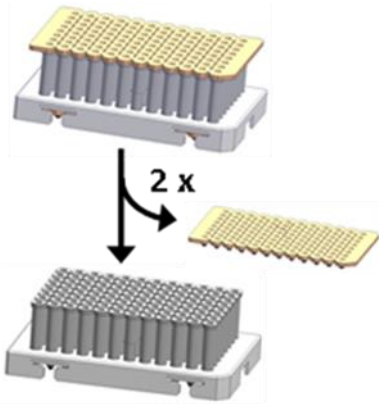
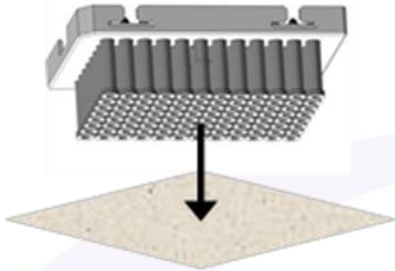


Protocol for Use

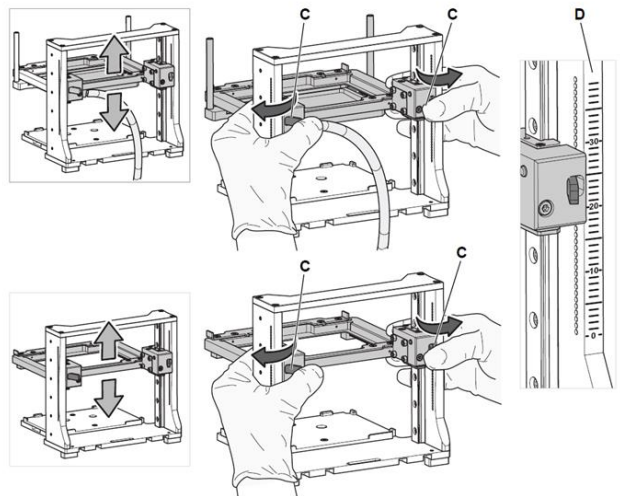
The below protocol is intended as a basic general example, and adjustments will be necessary depending on the application. Refer to the Te-Chrom™ Wizard Software Manual from Tecan for further information.

To operate the RoboColumn units safely and effectively, the user must be familiar with the use of robotic workstations and have background knowledge of chromatography. The Freedom EVO® workstation should be equipped with Te-Chrom™ Module and Te-Chrom™ Shuttle.

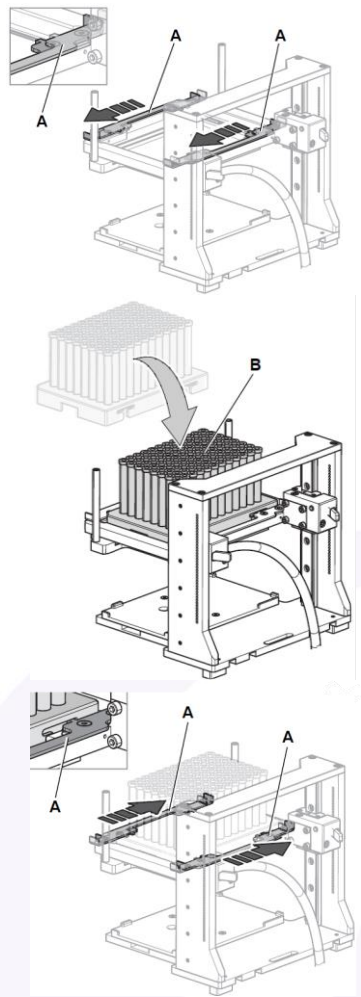
Setting up the RoboColumn array plate:

Step	Instruction	Image
1.	Remove the bottom cover seal and arrange rows of RoboColumns in a 96-well array plate. Remove the top seals.	
2.	Remove excess storage buffer by blotting the 96-well array plate upside down on tissue paper.	


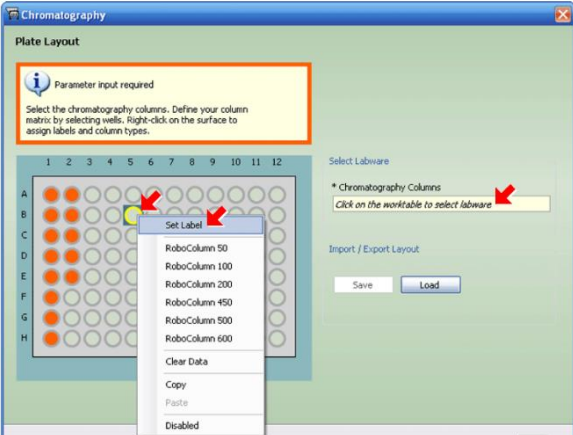
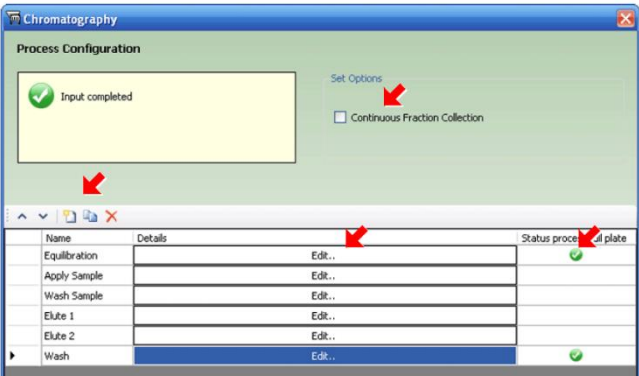

- 3 . Adjust the operating height of the Te-Chrom™ to the required height of the module scale (D) using the fastener (C).
 Try to make sure that the RoboColumns are positioned as close to collection plate as possible for the experiments.




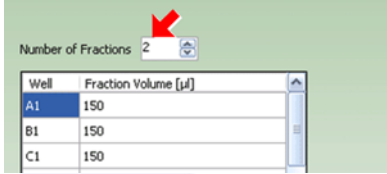


4. Load the RoboColumn array plate into the Te-Chrom™ by moving the locking slider to the left mechanical stop (A), loading the plate (B), and moving the locking slider back to the right (A).

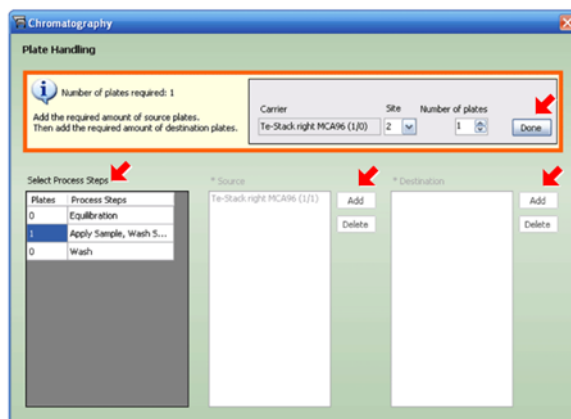


Setting up the Te-Chrom™ Wizard:

Step	Instruction	Image																												
1.	<p>Configure the Te-Chrom™ hardware by selecting the appropriate configuration and options, and defining a transfer position for the collection plate.</p>																													
2.	<p>Configure the plate layout by selecting the RoboColumn labware, defining the matrix and dimensions of the columns, labelling the samples appropriately, and importing/exporting the plate layout.</p>																													
3 .	<p>Define the workflow by setting individual steps using the Te-Chrom™ Wizard Process Configuration. The Continuous Fraction Collection option allows fractions to be collected over multiple plates without leaving gaps between process steps to optimise the use of collection plates.</p>	 <table border="1" data-bbox="708 1503 1350 1653"> <thead> <tr> <th>Name</th> <th>Details</th> <th>Status process</th> <th>All plate</th> </tr> </thead> <tbody> <tr> <td>Equilibration</td> <td>Edit..</td> <td></td> <td>✓</td> </tr> <tr> <td>Apply Sample</td> <td>Edit..</td> <td></td> <td></td> </tr> <tr> <td>Wash Sample</td> <td>Edit..</td> <td></td> <td></td> </tr> <tr> <td>Elute 1</td> <td>Edit..</td> <td></td> <td></td> </tr> <tr> <td>Elute 2</td> <td>Edit..</td> <td></td> <td></td> </tr> <tr> <td>Wash</td> <td>Edit..</td> <td></td> <td>✓</td> </tr> </tbody> </table>	Name	Details	Status process	All plate	Equilibration	Edit..		✓	Apply Sample	Edit..			Wash Sample	Edit..			Elute 1	Edit..			Elute 2	Edit..			Wash	Edit..		✓
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4.	<p>Define the parameters for each workflow process step: a) Process step Define the step name, and select 'Process Full Plate' to process the full 99-column array plate. If this is not selected, the workflow set out in step 3 will be carried out individually column by column.</p>																													

<p>b) Liquid transfer Set wash tips command, volume, and liquid handling parameters according to requirements. Set the dispensing volume, liquid class for aspiration, and flow rate (cm/h). The residence time can be calculated by dividing the column volume by the volumetric flow rate (mL/min).</p>									
<p>c) Waste option Select 'Engage Waste Tray' to collect flow-through in a waste tray. If this isn't selected, the flow-through will be collected in a collection plate.</p>									
<p>d) Neutralization and data collection Select 'Neutralization' to perform a pH adjustment after acidic elution. Select 'Data Collection' if it is necessary to evaluate the collection plate in a plate reader device, e.g., UV measurement.</p>									
<p>e) Fractions If 'Engage Waste Tray' is not selected (see point c), above), enter the number of fractions to collect for each chromatography step.</p>	 <table border="1" data-bbox="719 1451 1011 1552"> <thead> <tr> <th>Well</th> <th>Fraction Volume [µl]</th> </tr> </thead> <tbody> <tr> <td>A1</td> <td>150</td> </tr> <tr> <td>B1</td> <td>150</td> </tr> <tr> <td>C1</td> <td>150</td> </tr> </tbody> </table>	Well	Fraction Volume [µl]	A1	150	B1	150	C1	150
Well	Fraction Volume [µl]								
A1	150								
B1	150								
C1	150								

5. For each workflow step, use the 'Plate Handling menu' to enter the required number of source plates and destination plates.



6. Use the 'Reporting' menu to select the output folder and format for the data files.

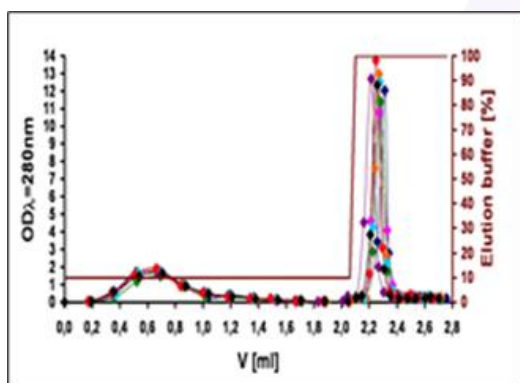


After the experimental set-up is complete, the Te-Chrom™ Wizard will generate a method in Freedom EVOware® software automatically.

Once all workflow parameters are defined, and the worktable is properly arranged, run the method, making sure that all required solvents and consumables (e.g., collection plates) are in the designated positions on the robotic worktable.

Evaluate the data by determining the precise fraction volumes and protein concentrations with an appropriate plate reader, using UV measurement for the protein concentrations. Use robotic arms to transfer the collection plate to another location on the worktable or to a secondary device for further analysis after path length detection.

Plot the UV data against the fraction volumes to create a pseudo-chromatogram of the run completed, making sure blank values of solvents are subtracted from the UV data before plotting. The plot may look similar to the below example, depending on the nature of the run being completed:



The RoboColumns can be regenerated using the conditions stated in the relevant chromatography media user guide. Recommended storage conditions can also be found in the relevant user guide, or in the Technical Characteristics table at the start of this brochure.

Troubleshooting

Problem	Possible causes	Actions
The RoboColumns are blocked/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 for 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.
The RoboColumns are leaking.	<ul style="list-style-type: none"> • Columns are clogged. • The o-ring seal is damaged. • The insertion depth of the robotic needle is incorrect. 	<ul style="list-style-type: none"> • U Refer to clogged actions listed above. • Check the taught X- and Y-coordinates for RoboColumn labware for precision. • Verify correct insertion depth of the robotic needle (16 mm from the top of the column).
The RoboColumns become separated from the 96-well array plate when the robotic needles are lifted.	<ul style="list-style-type: none"> • The RoboColumns are not securely fixed into the 96-well array plate. 	<ul style="list-style-type: none"> • Ensure each row of RoboColumns is securely mounted in the 96-well array plate prior to use (an audible click indicates that columns are securely in place).
Low protein recoveries in the elution fractions.	<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 to increase the residence time.

ORDER INFORMATION

Code	Description	Pack Size
4520-PC3450	CM PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3451	DEAE PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3452	Q PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3453	SP PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3408	Phenyl PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3416	Butyl PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3426	Hexyl PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3455	Octyl PuraBead® HF RoboColumns, 200 µL	Row of 8
4520-PC3250	EtoxiClear® RoboColumns, 200 µL	Row of 8
4520-PC3904	Fabsorbent™ F1 HF RoboColumns, 200 µL	Row of 8
4520-PC3160	Insulin Adsorbent RoboColumns, 200 µL	Row of 8
4520-PC0320	p-Aminobenzamidine A6XL RoboColumns, 200 µL	Row of 8
4520-PC9501	PE PuraBead® 6HF RoboColumns, 200 µL	Row of 8
4520-PC3260	HCPure™ RoboColumns, 200 µL	Row of 8

96-well array plate and additional cover seals can be purchased directly from Repligen GmbH.



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