RoboColumn®
Instruction Manual

TABLE OF CONTENTS

TOSOH BIOSCIENCE

RoboColumn Instruction Manual

1. INTRODUCTION

RoboColumns are miniaturized chromatography columns pre-packed with TOYOPEARL biopurification chromatography resins covering a wide range of chromatographic modes, such as ion exchange (IEX), hydrophobic interaction chromatography (HIC), multimodal chromatography (MMC), gel filtration (SEC) and affinity chromatography (AFC). 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.

RoboColumns are compatible with 96-well formats and designed for fully automated chromatographic separation with robotic liquid handling workstations such as the Freedom EVO® from Tecan. The column units are packed with TOYOPEARL® media by Repligen GmbH and are identical to the MediaScout® RoboColumns® supplied by Repligen GmbH. One package contains eight pre-packed RoboColumn units with two removable silicon cover seals for proper storage. A 96-well array plate is available to arrange up to 96 RoboColumn units according to application requirements.

APPLICATIONS

RoboColumn technology is used in a broad spectrum of applications, such as parallel screening of chromatographic media 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. It can also be applied for high throughput small scale purifications of a high number of samples.

FORMATS

RoboColumns are available in two formats with 200 µL and 600 µL resin volume, respectively. All chromatographic media used in the RoboColumns are also available as bulk resins for use at all scales.

2. CHARACTERISTICS

RoboColumns are ready-to-use chromatography columns pre-packed with TOYOPEARL chromatography media. RoboColumns are available in 200 µL and 600 µL volume. Table 1 presents the column geometries and characteristics of RoboColumns.

➤ **TABLE 1**

RoboColumn Chracteristics

Column volume	200 µL	600 µL
Bed height	10.0 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	AIEX and CIEX: 20% ethanol + 150 mmol/L NaCl HIC, SEC, AFC and MMC: 20 % ethanol	
Recommended storage temperature	IEX, HIC & MMC Media: +4°C to +30°C AFC Media: +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	

[1] Note: The chemical stability of the packed chromatography media also has to be taken into consideration. For more information please refer to the specification of the respective TOYOPEARL resin at www.tosohbioscience.de.

RoboColumn Instruction Manual

3. HANDLING AND OPERATION

A) 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.

B.) SAMPLE PREPARATION

We recommend loading a clarified sample on RoboColumns, since unclarified sample may cause clogging of the top and bottom filter frits.

TABLE 2

RECOMMENDED EQUIPMENT FOR AUTOMATED HANDLING OF RoboColumns
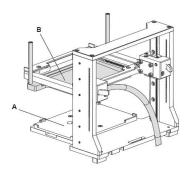
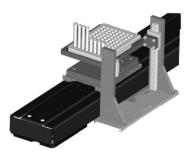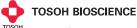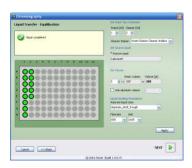| EQUIPMENT	DETAILS	ADVICE
96-WELL ARRAY PLATE (Courtesy of Repligen GmbH)	Arrange up to 96 individual Robo-Columns onto the 96- well array plate according to application requirements.	➤ Do not mix short (200 µL) and long (600 µL) RoboColumns. ➤ Make sure that RoboColumns are well clicked into the 96- well array plate before using them.
Te-Chrom™ MODULE[1] (Courtesy of Tecan)	Use the Te-Chrom module (A) to integrate the RoboColumn array onto the robotic worktable. The Te-Chrom Module is height adjustable and contains a waste option (B)	➤ Use the Te-Chrom module in combination with the plate stacker Te-Stack™ for the optimized handling and storage of collection plates. ➤ Use the waste container to drain off e.g. conditioning or regeneration solvents. ➤ Make sure that the array is in an appropriate distance to the collection plate before starting the experiment.
Te-Chrom SHUTTLE (Courtesy of Tecan)	Use the Te-Chrom Shuttle to move a collection plate under the array to collect the fractions.	➤ Use the robotic manipulator (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 or auto sampler) for further analysis such as UV measurement, ELISA, HPLC, MS, etc.

RoboColumn Instruction Manual

Te-Chrom WIZARD[2]

(Courtesy of Tecan)

Use the Te-Chrom Wizard to configure the chromatographic process and to set all process relevant parameters, such as 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 fractions after acidic elution of mAbs.

Note: Confirm suitability for your specific application with Tecan.

PLATE READER

Use an internal or external plate reader for data collection.

➤ Determine the precise volume of your 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 96-well half area micro-plate to collect fractions in a range from 25 µL to 175 µL.

➤ Use standard 96-well micro-plates to collect fractions in a range from 75 µL to 340 µL. Use 96-deep well reservoirs to collect fraction up to 2 mL.

[1] For more detailed information about the Te-Chrom Module, see Te-Chrom Operating Manual from Tecan

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

RoboColumn Instruction Manual

C. EXPERIMENTAL SETUP

RoboColumns are suitable for a broad variety of applications such as parallel screening and optimization of chromatographic conditions in downstream process development, sample preparation prior to subsequent analysis as well as the concentration of low titer compounds in drug discovery.

When using the high-throughput process development approach for RoboColumns, it is suggested to use Design of Experiments (DoE) for the experimental setup. This enables screening of many different chromatographic parameters, such as resin type, pH, conductivity/ionic strength or salt type simultaneously.
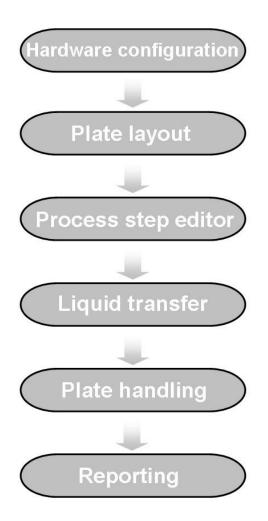
For experimental set-up and configuration of the RoboColumn array plate, the Te-Chrom Wizard is recommended.
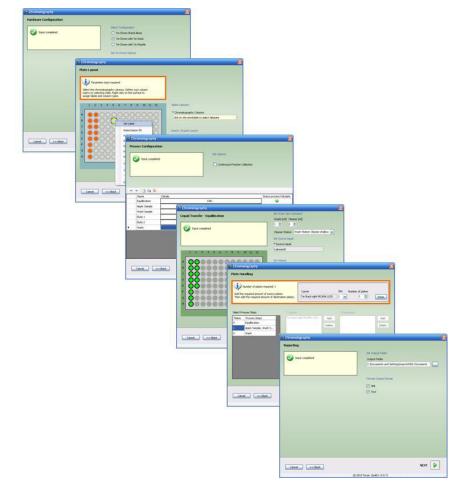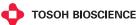
D. Te-Chrom WIZARD

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 and to set some chromatographic parameters, such as flow-rate or volume without direct script writing. See the illustration below for an overview of some of the Te-Chrom Wizard functions.

➤ **FIGURE 18**

Te-Chrom WIZARD FEATURES

RoboColumn Instruction Manual

4. PROTOCOLS

The protocol is designed as a general guideline for working with TOYOPEARL RoboColumns. Adjustment may be required depending on type of application, sample, resin type and RoboColumn dimension.

In order to operate RoboColumns the Freedom EVO workstation should be equipped with Te-Chrom Module and Te-Chrom Shuttle.
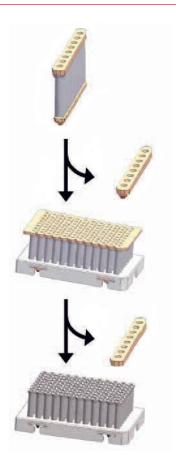
The user should have a general knowledge of chromatography and should be familiar with the use of robotic workstations.

A) DETAILED PROTOCOL

Follow the actions described below in order to perform a complete experiment.
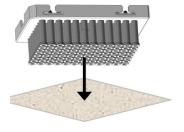
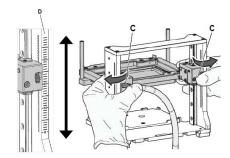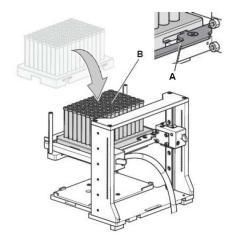| STEP	ILLUSTRATION	ACTION
1		**REMOVE COVER SEALS** ➤ Gently peel off the bottom seal from the row of eight RoboColumn units. ➤ Assemble the Robocolumn units onto the 96-well array plate. Up to 12 rows of eight RoboColumn units can be assembled. Do not mix different formats (200 µl and 600 µl) on the same array plate. ➤ Gently peel off the top seal or each row of eight RoboColumns.
2		**REMOVE EXCESS STORAGE SOLUTION** ➤ Remove remaining storage solution in the top part of the columns by blotting the RoboColumn® array upside down on a soft paper tissue.

RoboColumn Instruction Manual

3

Te-Chrom - ADJUST 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).

➤ *Make sure that the RoboColumn array is as close to the collection plate as possible.*

4

Te-Chrom - LOADING THE 96-WELL ARRAY EQUIPPED WITH RoboColumns

➤ Move the locking slider (A) to the left mechanical stop.

➤ Load the RoboColumn array (B).

➤ Move the locking slider (A) to the right mechanical stop to lock the RoboColumn array.

➤ *Make sure that all RoboColumns are well fixed into the 96-well array plate before starting the experiment.*

5

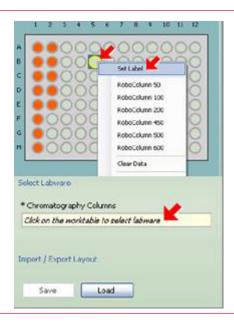Te-Chrom WIZARD – EXPERIMENTAL SETUP

A) Hardware configuration

➤ Select the appropriate configuration of the Te-Chrom

➤ If the Te-Chrom is equipped with a waste tray, check the 'Waste Tray' box.

➤ Define a transfer position for the collection plate or check the 'manual plate handling' box to select manual plate handling.

RoboColumn Instruction Manual

B) PLATE LAYOUT

➤ Click load to import an existing plate layout. To create a new layout, follow the actions below:

➤ Select the RoboColumn labware.

➤ Define the required RoboColumn matrix.

➤ Select the RoboColumn dimension.

➤ Set a desired label e.g. resin name.

➤ To import or export the plate layout, click the save button.

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. This minimizes the consumption of collection plates.
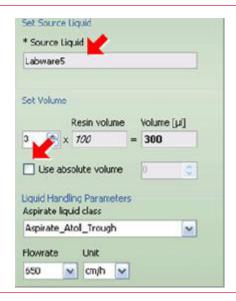

D) CONFIGURE YOUR PROCESS STEPS

➤ Edit a 'process step name' e.g. Equilibration:

➤ Check the 'Process Full Plate' option according to the desired procedure:

➤ When checked: The full RoboColumn array will be processed.

➤ When not checked: The RoboColumn array will be processed column per column.

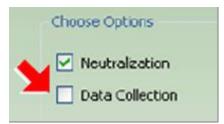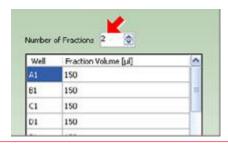➤ Set parameters for tip washing.

RoboColumn Instruction Manual

➤ Set individual chromatographic parameters:

➤ Select the source of liquid.

➤ Set the dispensing volume or check the 'Use absolute volume' box to enter an absolute volume.

➤ Set the required liquid class for aspiration.

➤ Set the required linear velocity [cm/h] or flow rate [µL/s].

➤ 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 e.g. after 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.

➤ Repeat these steps in order to configure all chromatographic process steps.

RoboColumn Instruction Manual

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.
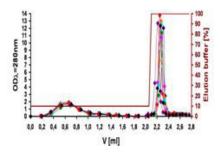
| 6 | | RUN CHROMATOGRAPHIC METHOD |

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.

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 the recommendation for the corresponding TOYOPEARL resin.

➤ For appropriate storage of RoboColumns please refer to Table 1

RoboColumn Instruction Manual

5. TROUBLESHOOTING

ERROR	POSSIBLE CAUSE	ACTION
RoboColumns are clogged.	The sample is too viscous.	Increase dilution of the cell paste before lysis, or dilute after the lysation.
	There is too much cell debris in the sample.	Centrifuge and/or filtrate the sample if unclarified sample has been used.
	The column has been regenerated and used too many cycles.	Reduce the number of cycles.
RoboColumns are leaking.	Columns are clogged.	See above.
	The o-ring sealing is damaged.	Check the taught X- and Y coordinates for RoboColumn labware for precision.
	The insertion depth of the robotic needle is incorrect.	Check the insertion depth (16 mm from 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.	RoboColumns are not well fixed into the 96-well array plate.	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).	The residence time for sample loading is too low.	Decrease the flow-rate (increase residence time) for sample loading.

RoboColumn Instruction Manual

➤ **ORDERING INFORMATION**

P/N	RoboColumn	Resin
RoboColumns FOR FAST AUTOMATED SCREENING OF RESINS		
45099	Array Plate	
GEL FILTRATION CHROMATOGRAPHY / DESALTING		
0045071	RoboColumn HW-40F	200 µL x 8 columns
0045072	RoboColumn HW-40F	600 µL x 8 columns
ION EXCHANGE CHROMATOGRAPHY		
0045027	RoboColumn Sulfate-650F,	200 µL x 8 columns
0045028	RoboColumn Sulfate-650F,	600 µL x 8 columns
0045023	RoboColumn GigaCap S-650S	200 µL x 8 columns
0045024	RoboColumn GigaCap S-650S	600 µL x 8 columns
0045001	RoboColumn GigaCap S-650M	200 µL x 8 columns
0045002	RoboColumn GigaCap S-650M	600 µL x 8 columns
0045025	RoboColumn GigaCap Q-650S	200 µL x 8 columns
0045026	RoboColumn GigaCap Q-650S	600 µL x 8 columns
0045003	RoboColumn GigaCap Q-650M	200 µL x 8 columns
0045004	RoboColumn GigaCap Q-650M	600 µL x 8 columns
0045005	RoboColumn GigaCap CM-650M	200 µL x 8 columns
0045006	RoboColumn GigaCap CM-650M	600 µL x 8 columns
0045007	RoboColumn GigaCap DEAE-650M	200 µL x 8 columns
0045008	RoboColumn GigaCap DEAE-650M	600 µL x 8 columns
0045021	RoboColumn NH$_2$-750F	200 µL x 8 columns
0045022	RoboColumn NH$_2$-750F	600 µL x 8 columns
MIXED MODE CHROMATOGRPAHY		
0045051	RoboColumn MX-Trp-650M	200 µL x 8 columns
0045052	RoboColumn MX-Trp-650M	600 µL x 8 columns
0045053	RoboColumn Ca^{++}Pure-HA,	200 µL x 8 columns
0045054	RoboColumn Ca^{++}Pure-HA,	600 µL x 8 columns

RoboColumn Instruction Manual

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

0045031	RoboColumn Phenyl-600M	200 µL x 8 columns
0045032	RoboColumn Phenyl-600M	600 µL x 8 columns
0045033	RoboColumn Butyl-600M	200 µL x 8 columns
0045034	RoboColumn Butyl-600M	600 µL x 8 columns
0045035	RoboColumn PPG-600M	200 µL x 8 columns
0045036	RoboColumn PPG-600M	600 µL x 8 columns
0045037	RoboColumn Phenyl-650M	200 µL x 8 columns
0045038	RoboColumn Phenyl-650M	600 µL x 8 columns
0045089	RoboColumn Butyl-650M	200 µL x 8 columns
0045090	RoboColumn Butyl-650M	600 µL x 8 columns
0045091	RoboColumn Hexyl-650C	200 µL x 8 columns
0045092	RoboColumn Hexyl-650C	600 µL x 8 columns

AFFINITY CHROMATOGRAPHY

0045065	RoboColumn AF-rProtein L-650F,	200 µL x 8 columns
0045066	RoboColumn AF-rProtein L-650F,	600 µL x 8 columns
0045063	RoboColumn AF-rProtein A HC-650F	200 µL x 8 columns
0045064	RoboColumn AF-rProtein A HC-650F	600 µL x 8 columns
0045061	RoboColumn AF-rProtein A-650F	200 µL x 8 columns
0045062	RoboColumn AF-rProtein A-650F	600 µL x 8 columns