Foresight™ RoboColumn Units 200 and 600 µl

Instruction Manual

Catalog Numbers

732-4801	732-4816
732-4802	732-4817
732-4804	732-4819
732-4805	732-4820
732-4807	732-4822
732-4808	732-4823
732-4813	732-4825
732-4814	732-4826
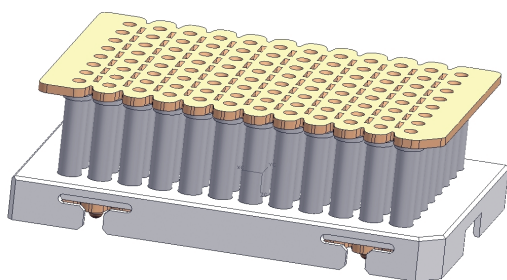
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Legal Notices

RoboColumn is a trademark of Atoll GmbH.

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Table of Contents

Section 1
Introduction

Foresight™ RoboColumn units are prepacked chromatography microcolumns used for rapid process development. Their design is fully compatible with robotic liquid handling workstations, such as the Freedom EVO series from Tecan, and allows process developers to perform high throughput (HT) parallel screening and experimentation. Foresight RoboColumn units may be used for qualitative media screening experiments and/or small scale methods development. Through design of experiments (DoE) utilizing Foresight RoboColumn units, an operational window can be defined to aid in developing a quality by design (QbD) process.

The design of the Foresight column offers:

1. **Convenience**

   The prepacked columns used in conjunction with a robotic liquid handling device allow users to create automated protocols for HT experimentation. The ability to define a design space enables process scientists to develop robust purification processes.

2. **Reliability**

   Each column is individually packed under optimum compression, ensuring consistent experimental results.

3. **Efficiency**

   The small column design enables users to use precious feed sample more efficiently while performing real chromatographic separations in parallel to determine critical quality parameters.

Foresight RoboColumn units are available in a variety of chromatography modes including anion, cation, and mixed-mode. See Section 6, Ordering Information, for the complete product line.

Section 2
Characteristics

Foresight™ RoboColumn units are ready-to-use chromatography microcolumns prepacked with Bio-Rad Laboratories process chromatography resins. Each row contains eight columns and either a single row or multiple rows may be used. For HT screening, configuration into 96-well plate format is possible with the use of an array plate.

Foresight RoboColumn units are available in two volumes: 200 and 600 µl.

Table 1. Characteristics of Foresight RoboColumn units.

Plate size	128.3 x 86.0 x 14.0 mm
Plate material	Polyoxymethylene copolymer
Number of wells	Polypropylene/polyethylene
Column inner diameter	5 mm
Column material	Polypropylene
Chemical stability	Stable in organic solvents. UNOsphere™ and Nuvia™ resins are stable in commonly used aqueous buffers pH 1–14. CHT™ resin is stable in aqueous buffers of pH >60.5.*
Avoid	Halogenated organic solvents, hexane
Storage solution	• UNOsphere Q, UNOsphere S, UNOsphere Rapid S, Nuvia™ cPrime™: 20% ethanol • CHT Type I, 40 µm and CHT Type II, 40 µm: 20% ethanol + 0.01M Na phosphate, pH 6.8 • Nuvia Q: 20% ethanol + 1M NaCl • Nuvia S: 20 % ethanol + 0.1M NaCl
Recommended storage temperature	4–30°C
Working temperature	4–30°C
Maximum working pressure	Up to 8.0 bar
Flow velocity	16–1000 cm/h^2
Laser label	Placed on every individual column body and containing: • Product number • Resin name • Column volume in µl

*Specifications for all Bio-Rad chromatography media may be found by visiting www.bio-rad.com/process and downloading their corresponding instruction manual.

Table 2. List of Bio-Rad chromatography media.

Chromatography Media	Mode	Instruction Manual Literature #
UNOsphere Q	Strong Anion	4110109
UNOsphere S	Strong Cation	4110109
UNOsphere Rapid S	Strong Cation	10010339
Nuvia Q	Strong Anion	10018215
Nuvia S	Strong Cation	10018215
Nuvia cPrime	Mixed Mode (Hydrophobic-Cationic)	10023853
CHT Type I, 40 µm	Mixed Mode (Metal Affinity-Cationic)	6086
CHT Type II, 40 µm	Mixed Mode (Metal Affinity-Cationic)	6086

Section 3
Advice on Handling

3.1 Equipment

Foresight™ RoboColumn units are designed for robotic handling. Table 3 is a guide to the equipment required for robotic handling of the units using Tecan's Freedom EVO workstation.

Table 3. Recommended equipment for automated handling of Foresight RoboColumn units.
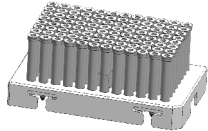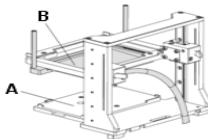
Equipment	Details	Tips and tricks
RoboColumn units 96-well array plate	Arrange up to 96 individual columns onto the 96-well array plate according to application requirements	• Do not mix short (200 µl) and long (600 µl) columns • Make sure the columns are securely seated into the 96-well array plate before using them
Te-Chrom Module (A)	Use the Te-Chrom module to integrate the Foresight RoboColumn units onto the robotic worktable • Height adjustable • Waste option (B)	• Use the Te-Chrom module in combination with a Te-Stack plate stacker for the optimized handling and storage of collection plates • Use the waste container to remove buffers from each step • Make sure that the columns are an appropriate distance from the collection plate before starting the experiment

Table 3. Recommended equipment for automated handling of Foresight RoboColumn units, continued.
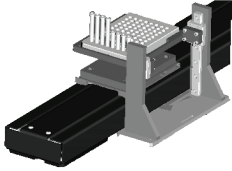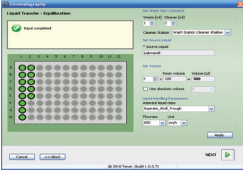
Equipment	Details	Tips and tricks
Te-Chrom Shuttle	Use the Te-Chrom shuttle to move a collection plate under the columns to fractionate the flowthrough from the column outlets	Use the Robotic Manipulator Arm (RoMa) to transport the collection plate from a predefined transfer position to any destination on the robotic worktable (plate reader, hotels, autosampler, etc.) for further analysis (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 (volume, flow rate, etc.)	• Assign labels (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 any specific application.
Plate Reader	Use the plate reader to evaluate data such as UV measurement and protein concentration	Calculate the precise volume of your collected fractions for subsequent data plotting

Table 3. Recommended equipment for automated handling of Foresight RoboColumn units, continued.

Equipment	Details	Tips and tricks
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
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 25–175 µl • Use a standard 96-well microplate to collect fractions in a volume range from 75–340 µl • Use 96-deep well reservoirs to collect high volume fractions up to 2.0 ml

3.2 Sample Preparation

Loading a clarified sample onto Foresight™ RoboColumn units is recommended because unclarified sample may cause clogging of the top and bottom filter frits. This may impact the performance of the column.

3.3 Experiment Setup

Foresight RoboColumn units are suitable for applications such as parallel screening and optimization of chromatographic conditions in downstream process development. The flexible design enables arrangement of up to 12 rows (8 columns per row) of Foresight RoboColumn units on a 96-well array plate (available upon request) according to application's requirements.

When using the HT process development approach for Foresight RoboColumn units, DoE is recommended for experiment setup, as it enables efficient and parallel screening of many different chromatographic parameters such as resin type, pH, conductivity or ionic strength, and salt type.

For experiment setup and configuration of the Foresight RoboColumn units, the Te-Chrom Wizard is recommended. See Section 3.4.

3.4 Te-Chrom Wizard

The Te-Chrom Wizard is a dialog-based graphical user interface offered by Tecan and based on the Freedom EVOware software.

The Te-Chrom Wizard was developed for use with Foresight RoboColumn units and enables configuration of hardware, plate layout, and setup of the chromatographic process, including relevant parameters such as flow rate and volume, without direct script writing (see Figure 1).

Please confirm with Tecan suitability for any specific application.

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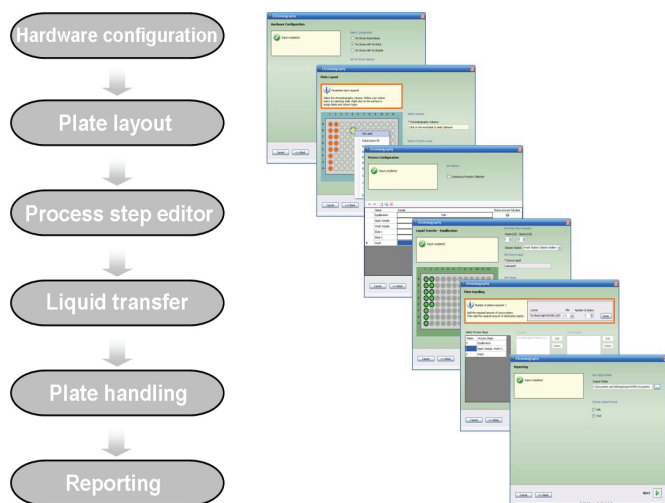

Fig. 1. Te-Chrom Wizard features.

Section 4
Protocol

The protocol is designed as a general guideline for working with Foresight™ RoboColumn units. Adjustment may be required depending on type of application, sample, resin type, and Foresight RoboColumn units dimensions.

One product package contains 8–96 individual prepacked Foresight RoboColumn units arranged on a 96-well array plate, and two removable cover seals for storage (see Figure 2).
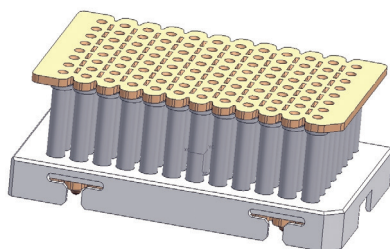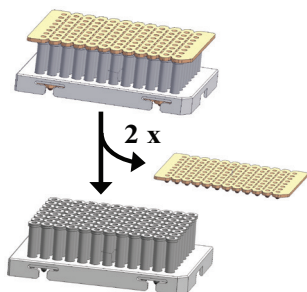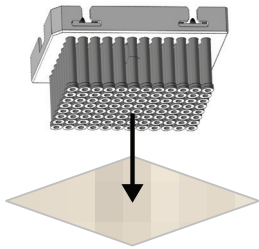

Fig. 2. 96 Foresight RoboColumn units.
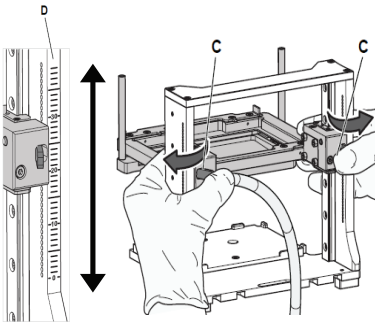
4.1 Detailed Protocol

1. Remove cover seals

    a. First, gently peel off the top seal from the columns

   b. Next, gently peel off the bottom seal from the columns

2. Remove excess storage solution from needle adaptor inlet

   Remove remaining storage solution in the top part of the columns by blotting them upside down on a soft paper tissue

3. To adjust the Te-Chrom module operating height, press and hold the fastener (C) and move the module to the required height of the scale (D)

   Note: Make sure that the columns are positioned as close as possible to the collection plate before starting the experiment
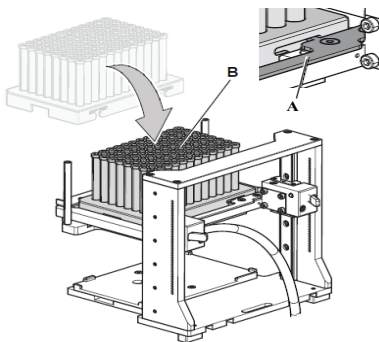
4. Loading the Foresight RoboColumn units in the Te-Chrom module

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

   b. Load the columns (B)

   c. Move the locking slider (A) to the right mechanical stop to lock the columns

   Note: Make sure that all columns are securely fixed into the array plate before starting the experiment.
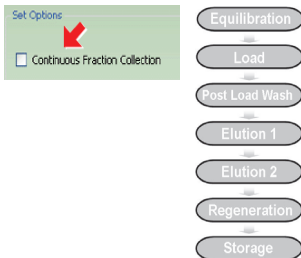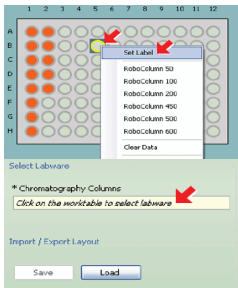
5. Experiment setup with the Te-Chrom Wizard

   a. Hardware configuration

   - Select the appropriate configuration of the Te-Chrom module
   - Check whether the module 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 such as 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, to prevent gaps between plates pipetted in different process steps and to optimize the consumption of collection plates

d. Configure your process steps

- Defiine a process step name such as Equilibration
- Check the Process Full Plate option if appropriate:
    i. When checked, the full array will be processed
    ii. When not checked, the array is processed column by column

- Set parameters for tip washing
- Set individual chromatographic parameters

    i. Select the liquid
    ii. Set the dispensing volume
    iii. Set the required liquid class for aspiration
    iv. Set the required flow rate (cm/h)

    Note: Calculate the required residence time (t) by dividing the column volume (CV) by the volumetric flow rate (U_v). $t = CV/U_v$
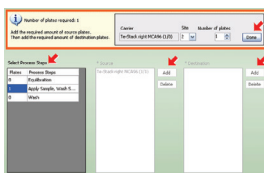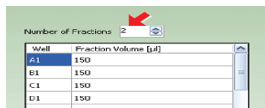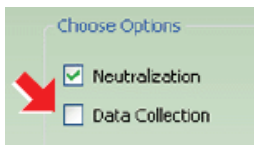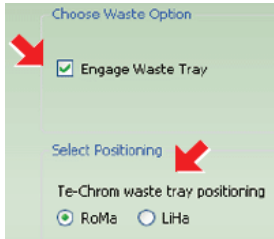
- Check the waste option if appropriate:
    i. When checked, the waste tray will be engaged by the RoMa or Liquid Handling Arm (LiHa)
    ii. When not checked, the flowthrough will be collected in a desired collection plate

- Check the Neutralization option to perform a pH adjustment, for example after acidic elution of mAbs
- Check the Data Collection option to evaluate the collection plate in a required reader device such as a UV reader

- 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

After the experiment setup the Te-Chrom Wizard will generate a chromatographic method in Freedom EVOware software automatically.
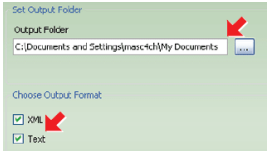
f. Reporting

- Set output folder
- Select output format

6. Run chromatographic method

   Note: Make sure all required solvents and consumables such as collection plates are in their designated positions on the robotic worktable before running the chromatographic method.

7. Data evaluation

   a. Determine the precise volume of the collected fractions by using an appropriate plate reader
   b. Determine the protein concentration by measuring UV

   Note: After path length detection with the RoMa, transfer the collection plate to any location on the robotic worktable, or to a secondary device for further analysis such as HPLC, MS, ELISA, or CGE/SDS-Page.
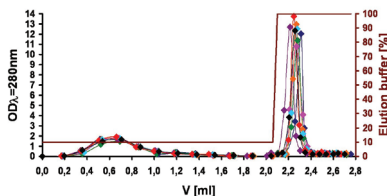
8. Data plotting

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

a. For appropriate regeneration of Foresight RoboColumn units please refer to www.bio-rad.com/process for the recommendation of the specific resin

b. For appropriate storage of Foresight RoboColumn units, refer to Table 1

Section 5
Troubleshooting Guide

Fault	Possible cause	Action
Foresight™ RoboColumn units are clogged	• 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	• Increase dilution of the cell isolate before lysis, or dilute after the lysation • Centrifuge and/or filter the sample if unclarified sample has been used • Reduce the number of cycles
Foresight RoboColumn units are leaking	• Columns are clogged • The O-ring seal is damaged • The insertion depth of the robotic needle is incorrect	• See above • Check the taught X and Y coordinates for RoboColumn labware for precision • Check the insertion depth (16 mm from the top of the column) of the robotic needle
Foresight RoboColumn units are lifted up from the 96-well array plate during the pull-out of the robotic needles	Columns are not securely seated into the 96-well array plate	Make sure that all columns are securely seated into the 96-well array plate by firmly pressing down 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 or increase residence time for sample loading

Section 6
Ordering Information

Foresight RoboColumn units 200 and 600 µl

Product	Mode	Catalog #
Foresight™ Nuvia™ S RoboColumn units, 200 µl	Strong Cation	732-4801
Foresight Nuvia S RoboColumn units, 600 µl	Strong Cation	732-4802
Foresight Nuvia Q RoboColumn units, 200 µl	Strong Anion	732-4804
Foresight Nuvia Q RoboColumn units, 600 µl	Strong Anion	732-4805
Foresight™ Nuvia™ cPrime™ RoboColumn units, 200 µl	Mixed Mode (Hydrophobic-Cationic)	732-4807
Foresight Nuvia cPrime RoboColumn units, 600 µl	Mixed Mode (Hydrophobic-Cationic)	732-4808
Foresight™ UNOsphere™ S RoboColumn units, 200 µl	Strong Cation	732-4813
Foresight UNOsphere S RoboColumn units, 600 µl	Strong Cation	732-4814
Foresight UNOsphere rS RoboColumn units, 200 µl	Strong Cation	732-4816
Foresight UNOsphere rS RoboColumn units, 600 µl	Strong Cation	732-4817
Foresight UNOsphere Q RoboColumn units, 200 µl	Strong Anion	732-4819
Foresight UNOsphere Q RoboColumn units, 600 µl	Strong Anion	732-4820
Foresight™ CHT™ Type I, 40 µm RoboColumn units, 200 µl	Mixed Mode (Metal Affinity-Cationic)	732-4822
Foresight CHT Type I, 40 µm RoboColumn units, 600 µl	Mixed Mode (Metal Affinity-Cationic)	732-4823
Foresight CHT Type II, 40 µm RoboColumn units, 200 µl	Mixed Mode (Metal Affinity-Cationic)	732-4825
Foresight CHT Type II, 40 µm RoboColumn units, 600 µl	Mixed Mode (Metal Affinity-Cationic)	732-4826

Foresight™ Chromatography Media Filter Plates

Product	Mode	Catalog #
Foresight™ Nuvia™ S Filter Plate, 20 µl	Strong Cation	732-4701
Foresight Nuvia Q Filter Plate, 20 µl	Strong Anion	732-4703
Foresight™ Nuvia™ cPrime™ Filter Plate, 20 µl	Mixed Mode (Hydrophobic-Cationic)	732-4705
Foresight™ UNOsphere™ S Filter Plate, 20 µl	Strong Cation	732-4710
Foresight UNOsphere rS Filter Plate, 20 µl	Strong Cation	732-4712
Foresight UNOsphere Q Filter Plate, 20 µl	Strong Anion	732-4714
Foresight™ CHT™ Type I, 40 µm Filter Plate, 20 µl	Mixed Mode (Metal Affinity-Cationic)	732-4716
Foresight CHT Type II, 40 µm Filter Plate, 20 µl	Mixed Mode (Metal Affinity-Cationic)	732-4718

Foresight™ Chromatography Media Columns

Product	Mode	Catalog #
Foresight™ Nuvia™ S Column, 1 ml	Strong Cation	732-4720
Foresight Nuvia S Column, 5 ml	Strong Cation	732-4740
Foresight Nuvia Q Column, 1 ml	Strong Anion	732-4721
Foresight Nuvia Q Column, 5 ml	Strong Anion	732-4741
Foresight™ Nuvia™ cPrime™ Column, 1 ml	Mixed Mode (Hydrophobic-Cationic)	732-4722
Foresight Nuvia cPrime Column, 5 ml	Mixed Mode (Hydrophobic-Cationic)	732-4742
Foresight™ UNOsphere™ S Column, 1 ml	Strong Cation	732-4730
Foresight UNOsphere S Column, 5 ml	Strong Cation	732-4750
Foresight UNOsphere rS Column, 1 ml	Strong Cation	732-4731
Foresight UNOsphere rS Column, 5 ml	Strong Cation	732-4751
Foresight UNOsphere Q Column, 1 ml	Strong Anion	732-4732
Foresight UNOsphere Q Column, 5 ml	Strong Anion	732-4752
Foresight™ CHT™ Type I, 40 µm Column, 1 ml	Mixed Mode (Metal Affinity-Cationic)	732-4735
Foresight CHT Type I, 40 µm Column, 5 ml	Mixed Mode (Metal Affinity-Cationic)	732-4755
Foresight CHT Type II, 40 µm Column, 1 ml	Mixed Mode (Metal Affinity-Cationic)	732-4736
Foresight CHT Type II, 40 µm Column, 5 ml	Mixed Mode (Metal Affinity-Cationic)	732-4756

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