OPUS® RoboColumn® Pre-packed Chromatography Columns

User Guide

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

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Abbreviations

AC	Affinity chromatography
AIEC	Anion exchange chromatography
cGE-PAGE	Capillary gel electrophoresis-polyacrylamide gel electrophoresis
CIEC	Cation exchange chromatography
DBC	Dynamic binding capacity
DoE	Design of Experiments
ELISA	Enzyme-linked immunosorbent assay
HCIC	Hydrophobic charge induction chromatography
HIC	Hydrophobic interaction chromatography
HPLC	High performance liquid chromatography
LC	Liquid chromatography
mAb	Monoclonal antibody
MMC	Mixed-mode chromatography
MS	Mass spectrometer
OPUS®	Open Platform, User Specified
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
τ	Residence time

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1.   Introduction

OPUS® RoboColumn® Pre-packed Columns (PPCs) are miniaturized chromatography columns that enable fully automated, parallel processing of chromatographic separations with a robotic liquid handling workstation such as the Tecan Freedom EVO® and the Tecan Fluent®.

OPUS® RoboColumn® PPCs are available pre-packed with over 300 user-specified resin types. Resin selection includes a wide variety of commercially-available chromatographic media with 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.

2.   About this document

This manual uses several different phrases. Each phrase should draw the following level of attention:

Table 1. Explanation of user attention phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

3.   Safety precautions

Table 2. Safety precautions for OPUS® RoboColumn® PPCs

Symbol		Description
WARNING	⚠	Wear standard laboratory PPE, including lab coat, protective eye wear, and gloves.
WARNING	⚠	This product is for laboratory use only. Not for administration to humans.
WARNING	🔥	Flammable liquid and vapor. • Keep away from heat/spark/open flame/hot surfaces. No smoking. • Keep container tightly closed. • Ground/bond container and receiving equipment. • Store in a well-ventilated place. Keep cool.
IMPORTANT	⚠	Dispose of contents/container in accordance with local/regional/national/ international regulations.

4.   Column description

OPUS® RoboColumn® PPCs can be configured in rows of eight (8) columns and easily arranged/fixed onto a 96-well array plate according to application requirements. As a result of this modular design, OPUS® RoboColumn® PPC 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 OPUS® RoboColumn® PPCs are also available in larger pre-packed OPUS® MiniChrom® PPCs 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.

4.1   Column components

OPUS® RoboColumn® PPC components are labeled below (Figure 1).

Figure 1. Column components: OPUS® RoboColumn® PPC
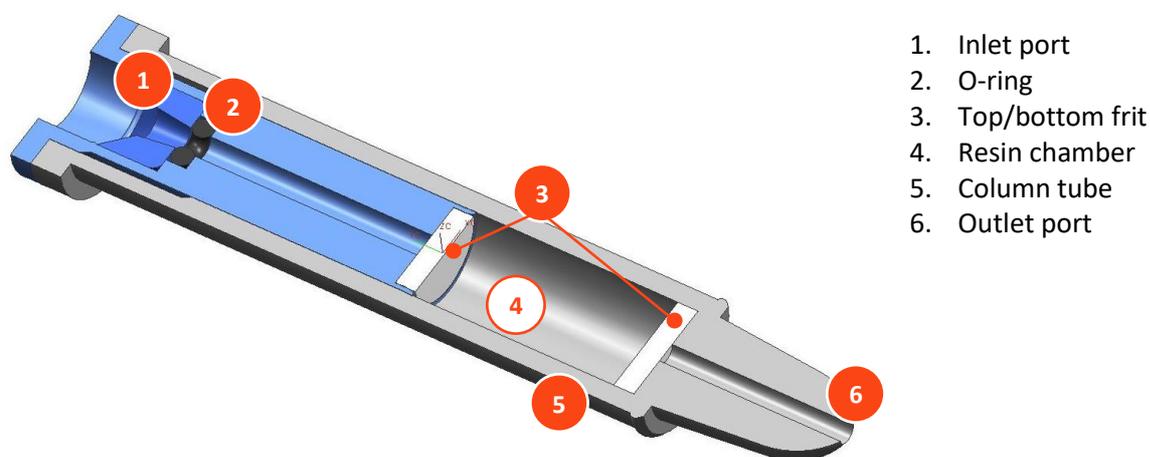
1. Inlet port
2. O-ring
3. Top/bottom frit
4. Resin chamber
5. Column tube
6. Outlet port

4.2   Column materials of construction

OPUS® RoboColumn® PPCs are manufactured using polymers compatible with bioprocess requirements (Table 3).

Table 3. Materials of construction

Component	Material
Array plate	Polyoxymethylene copolymer
O-ring	Viton®
Column tube	Polypropylene
Frit	Polypropylene/Polyethylene, 7 – 12 µm pore size (Porex®)

4.3 Column physical specifications

Table 4. Physical specifications summary

Physical attribute	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					
Plate size (96-well array)	128.3 x 86.0 x 14.0 mm					
Column label	Each individual column is laser labeled with the following: • Product number • Resin name • Column volume in µL					

4.4 Solvent compatibility

OPUS® RoboColumn® PPC product contact materials of construction consist of polyethylene and polypropylene. The column materials of construction are chemically stable when exposed to all commonly used aqueous buffers and organic solvents, pH 1 – 14.

> ⚠ **IMPORTANT:** Avoid halogenated organic solvents and the non-halogenated organic solvent hexane.

For information on the chemical stability of the packed chromatography media, refer to the manufacture's resin specification.

4.5 Column use recommendations

Table 5. Column use recommendations

Description	Recommendation
Storage solution	• AIEX, CIEX: 20% ethanol + 150 mM sodium chloride • HIC, SEC, AC, MMC: 20% ethanol • CHT™, CFT™ (Bio-Rad®): 20% ethanol + 10 mM sodium phosphate, pH 6.8 • HyperCel™ (Sartorius®): 30% isopropanol + 0.1 M sodium phosphate, pH 4.3 • ProSep® (Millipore®): 1% benzyl alcohol + 0.1 M sodium acetate, pH 5
Recommended storage temperature	• AIEX, CIEX, HIC, SEC, MMC: 4°C - 30°C • AC: 4°C - 8°C
Working temperature	4°C - 30°C
Maximum working pressure	8.0 bar
Flow velocity	16 - 1000 cm/h [‡]

[‡] Utilize the Liquid Transfer menu of the Te-Chrom™ Wizard 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).

5. Preparing to use OPUS® RoboColumn® PPCs

5.1 Equipment

OPUS® RoboColumn® PPCs are designed for use on a robotic liquid handler. Table 6 outlines the equipment required for robotic handling of OPUS® RoboColumn® PPCs using a Tecan Freedom EVO® workstation.

Table 6. Recommended equipment for automated handling of OPUS® RoboColumn® PPCs

Equipment	Details	Tips and tricks
OPUS® RoboColumn® PPC 96-well base plate	Arrange up to 96 individual OPUS® RoboColumn® PPCs onto the 96-well array plate according to application requirements.	• Do not mix short (50, 100, 200 µL) and long (450, 500, 600 µL) OPUS® RoboColumn® PPCs. • Ensure each OPUS® RoboColumn® PPC is securely mounted in the 96-well array plate prior to use (an audible click indicates that columns are securely in place).
Te-Chrom™ module	Use the Te-Chrom™ module to integrate the OPUS® RoboColumn® PPC array onto the robotic worktable. • Height adjustable • Waste option	• 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 OPUS® RoboColumn® PPC 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 OPUS® RoboColumn® PPC array to fractionate the flow-through coming from each column's outlet.	• 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.	• 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.	• 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.	• 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).	• 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 fraction up to 2.0 mL.

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5.2   Experimental set-up

Design of Experiments (DoE) is an ideal tool for developing robust, high-throughput assays. When used in combination with OPUS® RoboColumn® PPCs, DoE enables efficient screening of chromatographic parameters such as resin type, pH, conductivity/ionic strength, salt type, etc. The Tecan Te-Chrom™ Wizard and FluentControl™ applications can be used to translate DoE into liquid handling instructions that are compatible with the respective Tecan workstation. For more detailed information on how to use the Te-Chrom™ Wizard, refer to Section 6.3.

Examples of experimental set-ups are described in OPUS® RoboColumn® PPC application notes; see OPUS® RoboColumn® PPC literature, Section 9.

5.3   Sample preparation

Clarify sample/s prior to loading on OPUS® RoboColumn® PPCs. Unclarified sample/s may clog the top and bottom filter frits, which negatively impacts the column lifetime.

# 6.   Protocol

This protocol is designed as a general guideline for working with OPUS® RoboColumn® PPCs. Adjustments may be required depending on type of application, sample, resin type, and OPUS® RoboColumn® PPC dimensions.

6.1   Prepare the OPUS® RoboColumn® PPC array

1.   Remove cover seals (Figure 2). Gently peel off the top and bottom seal from the OPUS® RoboColumn® PPC array.
   - One product package contains 8 - 96 individual pre-packed OPUS® RoboColumn® PPCs arranged on a 96-well array plate and two removable cover seals for the appropriate storage of the OPUS® RoboColumn® PPC, see Figure 2.

Figure 2. Prepare OPUS® RoboColumn® PPC array: Remove cover seals
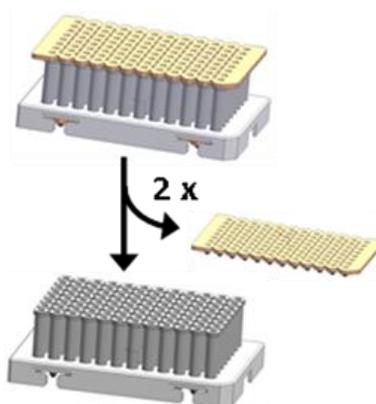
2. Remove excess storage solution from needle adaptor inlet (Figure 3). Remove remaining storage solution in the top part of the columns by blotting the OPUS® RoboColumn® PPC array upside down on a soft paper tissue.

Figure 3. Prepare OPUS® RoboColumn® PPC array: Remove excess storage solution
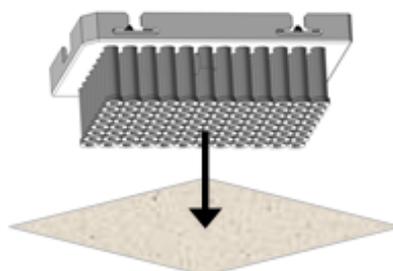
6.2   Prepare the Te-Chrom™ module

1. Adjust the operating height of the Te-Chrom™ (Figure 4). Press and hold the fastener (A) to set the Te-Chrom™ to the required height of the Te-Chrom™ module scale (B).

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

Figure 4. Adjust Te-Chrom™ operating height
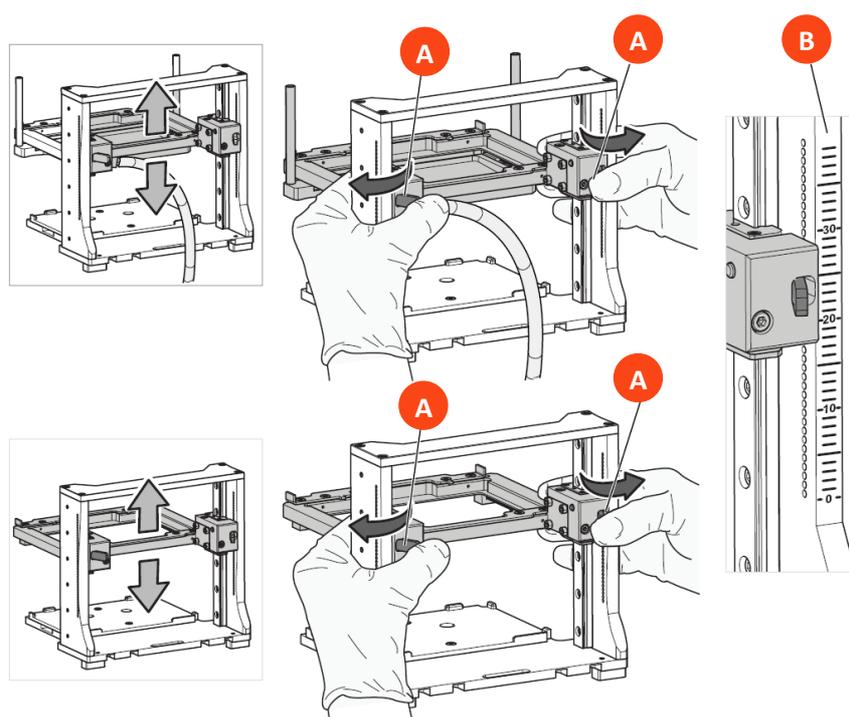
2. Load OPUS® RoboColumn® PPC array into Te-Chrom™ (Figure 5). Move the locking slider (A) to the left mechanical stop (Figure 5a). Load the OPUS® RoboColumn® PPC array (B) (Figure 5b). Move the locking slider (A) to the right mechanical stop to lock the OPUS® RoboColumn® PPC array in place (Figure 5c).

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

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Figure 5. Load OPUS® RoboColumn® PPC array into Te-Chrom™
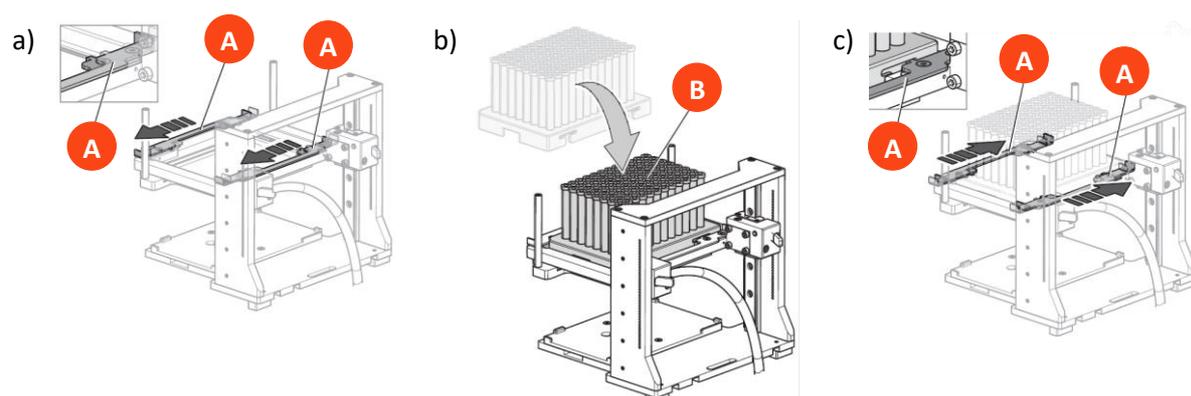
6.3 Set-up Te-Chrom™ Wizard

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 use with OPUS® RoboColumn® PPCs 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., flowrate and volume) without direct script writing (Figure 6).

Figure 6. Te-Chrom™ Wizard features
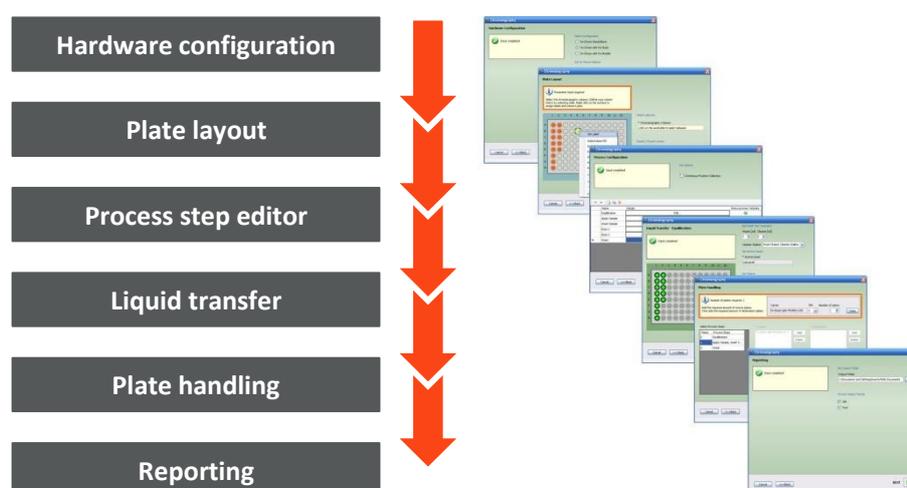
For more detailed information please refer to the Te-Chrom™ software manual. Consult with Tecan for application-specific needs.

1. Configure Te-Chrom™ hardware (Figure 7).
   - Select the appropriate configuration: Te-Chrom™ Stand Alone, Te-Chrom™ with Te-Stack, Te-Chrom™ with Te-Shuttle.
   - Select Te-Chrom™ options (e.g., waste tray).
   - Define a transfer position for the collection plate.

Figure 7. Te-Chrom™ Wizard Hardware Configuration menu

2. Configure plate layout in Te-Chrom™ Wizard (Figure 8).
    - Select the OPUS® RoboColumn® PPC labware.
    - Define the required OPUS® RoboColumn® PPC matrix.
    - Select the OPUS® RoboColumn® PPC dimension.
    - Label sample as desired, e.g., resin name.
    - Import or export the plate layout.

Figure 8. Te-Chrom™ Wizard Plate Layout menu
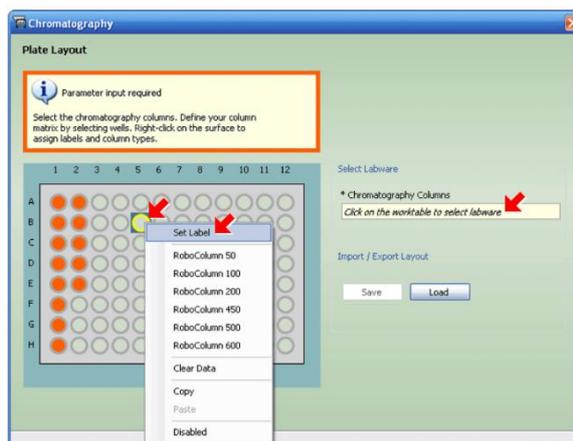
3. Define chromatographic workflow by setting individual steps using Te-Chrom™ Wizard Process Configuration (Figure 9).
    - Use the Continuous Fraction Collection option to collect fractions over multiple plates, without leaving gaps between plates pipetted in different process steps and to optimize the consumption of collection plates.

Figure 9. Te-Chrom™ Wizard Process Configuration menu
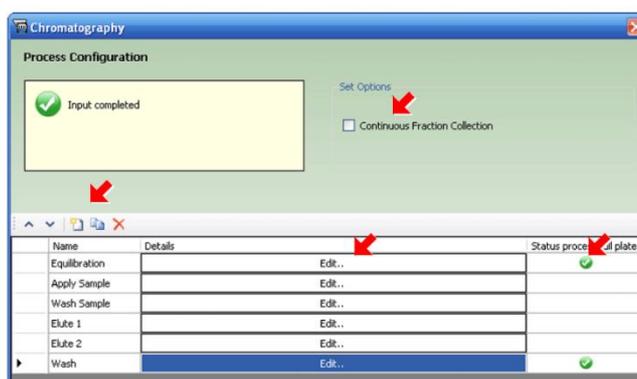
4. Define workflow parameters for each individual process step (Figure 10).

Figure 10. Define workflow parameters in Te-Chrom™ Wizard

Process Step
*   Define process name as desired, e.g. Equilibration.
*   Select "Process Full Plate" to process the full OPUS® RoboColumn® PPC array.
    o   If Process Full Plate is not selected, the chromatographic protocol defined in Step 3 will be executed column-by-column.

Liquid Transfer
*   Set wash tips command.
*   Set volume.
*   Set liquid handling parameters:
    o   Set the dispensing volume.
    o   Set the required liquid class for aspiration.
    o   Set the required flowrate [cm/h].
    o   Calculate the required residence time (τ) by dividing the OPUS® RoboColumn® PPC volume (CV) by the volumetric flowrate (uv): τ = CV/uv.

Waste Option
*   Select Engage Waste Tray to collect flow-through in a waste tray using robotic arms (e.g., RoMa, LiHa).
    o   When Engage Waste Tray is not selected, flow-through will be collected in a collection plate.

Neutralization and Data Collection
*   Select Neutralization to perform a pH adjustment after acidic elution of mAbs.
*   Select Data Collection to evaluate the collection plate in a plate reader device, e.g., UV measurement.
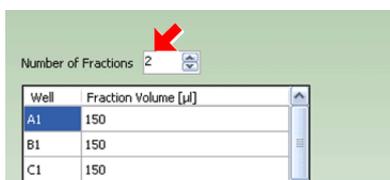
Fractions
*   If Engage Waste Tray is not selected (see Waste Option, above), enter the number of fractions to collect for each chromatography step.

5. For each process or process group, utilize the Plate Handling menu ([Figure 11](#)) to:
    - Enter the required number of source plates.
    - Enter the required number of destination plates.

Figure 11. Te-Chrom™ Wizard Plate Handling menu
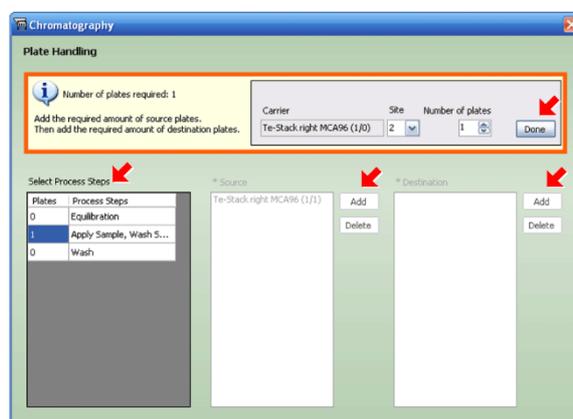
6. Utilize the Reporting menu ([Figure 12](#)) to:
    - Select output folder.
    - Select output format.

Figure 12. Te-Chrom™ Wizard Reporting menu
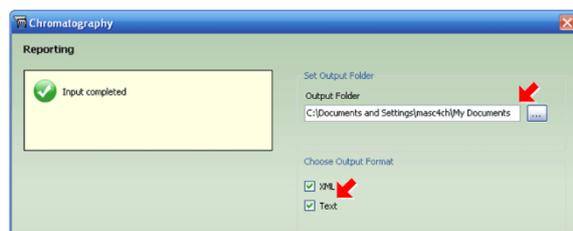
After the experimental set-up is complete, the Te Chrom™ Wizard will generate a chromatographic method in Freedom EVOware® software automatically.

6.4   Run chromatographic method

Once all workflow parameters are defined, and the worktable is properly arranged, run the 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.

6.5   Evaluate data

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

Note: Use robotic arms (e.g., RoMa, LiHa) to 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.

6.6   Plot data

Plot the UV data against the fraction volume to create a pseudo-chromatogram (Figure 13).

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

Figure 13. Te-Chrom™ Wizard data plotting example
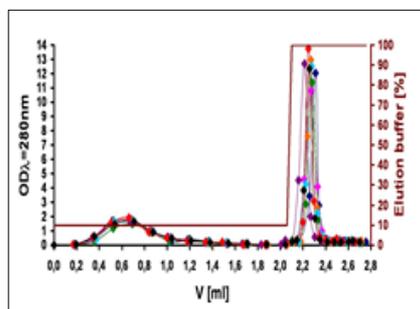
6.7   Regenerate resin and store the column array

- For appropriate regeneration of OPUS® RoboColumn® PPC, refer to manufacture's recommendation for the corresponding resin.
- For appropriate storage of OPUS® RoboColumn® PPC, refer to Table 5.

7.   Troubleshooting

OPUS® RoboColumn® PPC is clogged.

Possible causes	Actions
• The sample is too viscous. • There is too much cell debris in the sample. • The column has been regenerated and used too many cycles.	• Increase dilution of the cell paste before lysis or dilute after the lysation. • Centrifuge and/or filtrate the sample if unclarified sample has been used. • Reduce the number of cycles.

OPUS® RoboColumn® PPC is leaking.

Possible causes	Actions
• Columns are clogged. • The o-ring seal is damaged. • The insertion depth of the robotic needle is incorrect.	• Refer to clogged actions listed above. • Check the taught X- and Y-coordinates for OPUS® RoboColumn® PPC labware for precision. • Verify correct insertion depth of the robotic needle (16 mm from the top of the column).

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OPUS® RoboColumn® PPC separated from 96-well array plate when robotic needles are lifted.

Possible causes	Actions
• OPUS® RoboColumn® PPC is not well fixed into the 96-well array plate.	• Ensure each OPUS® RoboColumn® PPC is securely mounted in the 96-well array plate prior to use (an audible click indicates that columns are securely in place). • Purchase OPUS® RoboColumn® PPC downholder from Tecan to retain OPUS® RoboColumn® PPC in array plate.

Low protein recovery in the elution fraction(s).

Possible causes	Actions
• Residence time for sample loading is too low	• Decrease the flow rate for sample loading

8.   Ordering information

Table 7. OPUS® RoboColumn® PPC ordering information

Product	Quantity	Part number
OPUS® RoboColumn® PPC 96-well array plate	1	00000507
Cover seals, Top, Bottom	1	00000008
OPUS® RoboColumn® PPC, 50 µL	8-row	XXXXXX08R-02
OPUS® RoboColumn® PPC, 100 µL	8-row	XXXXXX08R-01
OPUS® RoboColumn® PPC, 200 µL	8-row	XXXXXX08R
OPUS® RoboColumn® PPC, 450 µL	8-row	XXXXXX08R-22.5
OPUS® RoboColumn® PPC, 500 µL	8-row	XXXXXX08R-25
OPUS® RoboColumn® PPC, 600 µL	8-row	XXXXXX08R-30

9.   Online literature

OPUS® RoboColumn® PPC literature can be found on the Repligen website.

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