

PreDictor RoboColumn

Instructions



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1 Introduction

PreDicator RoboColumn

PreDicator™ RoboColumn™ units are miniaturized chromatography columns prepacked with Cytiva BioProcess™ chromatography media. PreDicator RoboColumn units are made for fully automated chromatographic separation with the robotic liquid handling workstation Freedom EVO™ from Tecan™. PreDicator RoboColumn units are compatible with 96-well formats and can be used in a broad spectrum of applications, see [Chapter 2 Applications, on page 5](#). The images in this document are supplied by Tecan.

One product package contains eight prepacked PreDicator RoboColumn units with two removable silicon cover seals for proper storage. An example of a PreDicator RoboColumn product package is shown below:



Modular design

Up to 96 PreDicator RoboColumn units can be arranged on a 96-well array plate. This modular design facilitates the setup of automated parallel experiments.

Production of PreDicator RoboColumn units

PreDicator RoboColumn units are prepacked with Cytiva BioProcess chromatography media by Repligen GmbH. PreDicator RoboColumn units are identical to the RoboColumn units supplied by Repligen GmbH.

Other available formats for Cytiva chromatography media

All chromatography media used in PreDicator RoboColumn units are also available in:

- Larger prepacked columns such as HiTrap™ or HiScreen™
- Bulk media for use at all scales

Most of the chromatography media used in PreDicator RoboColumn units are also available in PreDicator 96-well filter plates for high-throughput screening.

2 Applications

Broad spectrum of applications

PreDicator RoboColumn units can be used in a variety of applications, see the Table below for examples.

Application	Description
Screening	Parallel screening of different chromatography media as well as different parts of the chromatographic cycle (e.g., binding, wash and elution). Parallel screening of different chromatographic conditions, for example pH, conductivity/ionic strength and type of salt.
Separation	In addition to isocratic separations it is possible to separate different compounds in a gradient elution by incremental elution steps.
Scale-down	Scale-down experiments.
Dynamic chromatographic parameters	Determination of dynamic binding capacity (DBC) by monitoring breakthrough curves. Determination of retention time (R_t) and retention volume (R_v).

3 Characteristics

Introduction

PreDicator RoboColumn units are miniaturized chromatography columns prepacked with Cytiva BioProcess chromatography media and compatible with 96-well formats. The chromatography media span a wide range of different methods, such as anion exchange (AEX), cation exchange (CIE), affinity chromatography (AC), hydrophobic interaction chromatography (HIC) and multimodal chromatography (MM).

Characteristics

The characteristics of PreDicator RoboColumn units are presented in the Table below.

Column volume	50 µl	200 µl	600 µl
Bed height	2.5 mm	10.0 mm	30.0 mm
Column height	39 mm	39 mm	59 mm
Column inner diameter	5 mm	5 mm	5 mm
Column material	Polypropylene		
Filter material	Polypropylene		
Filter pore size	7 to 12 µm		
Chemical stability	All commonly used aqueous buffers, pH 1 to 14, organic solvents ¹		
Avoid	• Halogenated organic solvents • Hexane		
Storage solution	• AEX, HIC, AC and MM chromatography media: 20% ethanol • CIE chromatography media: 20% ethanol + 0.2 M sodium acetate		
Recommended storage temperature	• AEX, CIE, HIC and MM chromatography media: 4°C to 30°C • AC chromatography media: 4°C to 8°C		
Working temperature	4°C to 30°C		

Maximum working pressure	Up to 8.0 bar for PreDicator RoboColumn units. For maximum working pressure for the chromatography medium, please refer to the characteristics for corresponding chromatography medium.
Flow velocity	16 to 1000 cm/h. For maximum working flow velocity for the chromatography medium, please refer to the characteristics for corresponding chromatography medium.

¹ **Note:** The chemical stability of the packed chromatography medium also has to be taken into consideration. For more information please refer to the specification for respective chromatography medium at [cytiva.com/bioprocess](https://www.cytiva.com/bioprocess).

4 Advice on handling

In this chapter

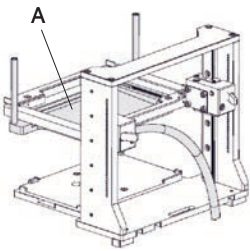
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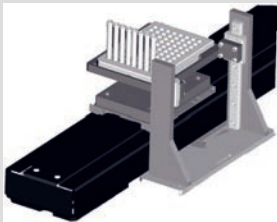
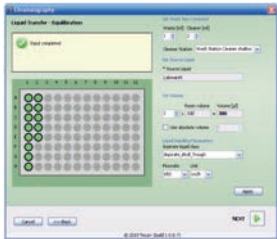
4.1 Equipment

Recommended equipment

PreDicator RoboColumn units are designed for robotic liquid handling only. The Table below is a guide for the equipment required for PreDicator RoboColumn units using Tecan's Freedom EVO workstation. Contact Tecan for details and options regarding the equipment.

Note: *The 96-well array plate can be ordered from Cytiva (28-9862-42).*

Equipment	Details	Advice on use
96-well array plate 	Arrange up to 96 individual PreDicator RoboColumn units onto the 96-well array plate according to application requirements.	• Do not mix short (50 µl, 200 µl) and long (600 µl) PreDicator RoboColumn units. • Make sure that the PreDicator RoboColumn units are well secured into the 96-well array plate before using them.
Te-Chrom Module¹ 	Use the Te-Chrom Module to integrate the 96-well array plate onto the robotic worktable. The Te-Chrom Module is height adjustable and contains a waste option (A).	• Use the Te-Chrom Module in combination with plate stacker Te-Stack for the optimized handling and storage of collection plates. • Use the waste container to drain off for example conditioning and regeneration solvents. • Make sure that the 96-well array plate is as close to the collection plate as possible before starting the experiment.

Equipment	Details	Advice on use
Te-Chrom Shuttle 	Use the Te-Chrom Shuttle to move a collection plate under the 96-well array plate to collect the fractions.	Use the robotic manipulator arm (RoMa) to transport the collection plate from a pre-defined transfer position to any destination on the robotic worktable (e.g., plate reader, hotels and auto sampler). This is useful in further analysis of the fractions by for example UV absorbance measurement, ELISA, HPLC and MS.
Te-Chrom Wizard² 	Use the Te-Chrom Wizard to configure the chromatographic process and to set all process relevant parameters, for example volume and flow velocity.	Assign labels (i.e., name of chromatography medium and type of column) by using the plate layout configurator.
Plate reader	Use an internal or external plate reader for data collection.	Determine the precise volume in the fractions.
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- or 96-well deep-well plate for buffer solution/preparation.

Equipment	Details	Advice on use
Collection plate	Use any type of multiwell plate which matches the ANSI/SDS standard (UV- or non UV-readable).	• Use a 96-well half area microplate to collect fractions in a volume range from 25 to 175 µl. • Use a standard 96-well microplate to collect fractions in a volume range from 75 to 340 µl. • Use 96-well deep-well plates to collect high volume fractions up to 2.0 ml.

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.

4.2 Sample preparation

Recommendations

It is recommended to load a clarified sample on the PreDicator RoboColumn, because an unclarified sample may cause clogging of the top and bottom filter frits. For information about sample preparation, see information at [cytiva.com/sampleprep](https://www.cytiva.com/sampleprep).

4.3 Experimental setup

Recommendations

When using the high-throughput process development approach for PreDicator RoboColumn units, it is suggested to use Design of Experiments (DoE) for the experimental setup.

For experimental setup and configuration of the 96-well array plate, the Te-Chrom Wizard is recommended.

4.4 Te-Chrom Wizard

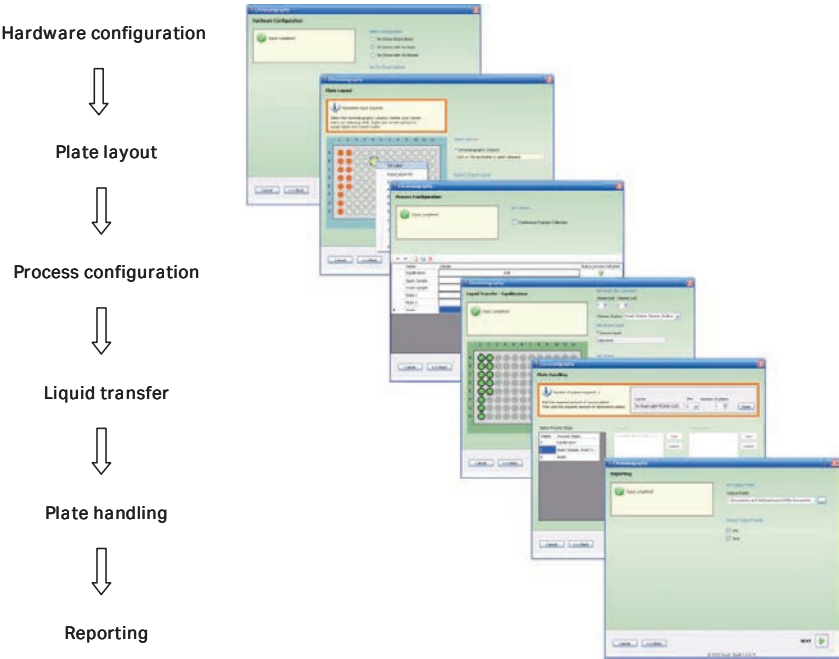
Introduction

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 is optional and facilitates the setup of a selection of chromatographic studies using PreDictor RoboColumn units. Confirm with Tecan for each application that the Te-Chrom Wizard can be used.

Features

The Te-Chrom Wizard provides the possibility to configure hardware, plate layout, the chromatographic process and to set all relevant chromatographic conditions. Some chromatographic parameters can be set without direct script writing, for example flow rate and volume.

See the illustration below for an overview of some of the Te-Chrom Wizard features.



5 Protocols

Introduction

The protocol is designed as a general guideline and adjustments may be required depending on type of application, sample and type of chromatography medium.

Prerequisites

In order to operate PreDicator RoboColumn units safely and according to the intended purpose the following prerequisites must be met:

- The Freedom EVO workstation should be equipped with Te-Chrom Module and Te-Chrom Shuttle.
- The user should be acquainted with the use of Tecan robotic workstations, including liquid handling.
- The user should have a general knowledge of chromatography.

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5.1 Te-Chrom Wizard - Experimental setup

Introduction

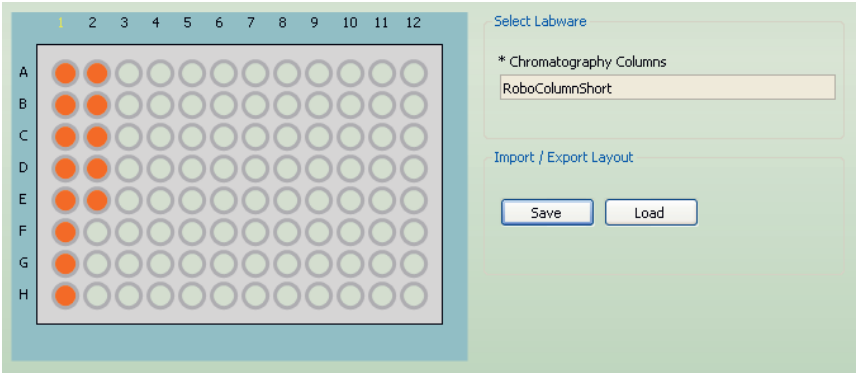
This section contains basic information of the main steps in the experimental setup. For more detailed information, see the *Te-Chrom Wizard Software Manual* from Tecan.

Hardware configuration

Follow the actions below to set the hardware configuration.

Step	Action
1	Select the appropriate configuration of Te-Chrom. Select Configuration ☐ Te-Chrom Stand Alone ☒ Te-Chrom with Te-Stack ☐ Te-Chrom with Te-Shuttle Select Te-Chrom Options ☒ Waste Tray Select Plate Transfer * Transfer position Te-Stack transfer/Te-Chrom (8/1) ☐ Manual plate handling
2	If Te-Chrom is equipped with a waste tray, then check the Waste Tray box.
3	Define a Transfer position for the collection plate or check the Manual plate handling box to select manual plate handling.

Plate layout



Click the **Load** button to import an existing plate layout. To create a new plate layout, follow the actions below.

Step	Action
1	Select the PreDicator RoboColumn labware.
2	Define the required PreDicator RoboColumn matrix.
3	Select the PreDicator RoboColumn dimension.
4	Set a desired label, for example the name of the chromatography medium.
5	To export the plate layout, click the Save button.

Process configuration

Follow the actions below to set the process configuration.

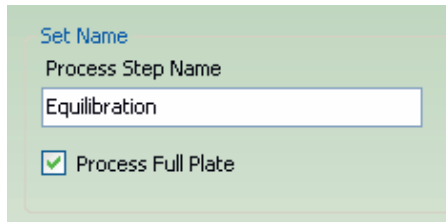
Step	Action
1	To collect fractions from different process steps without leaving gaps between the collected fractions, check the Continuous Fraction Collection box. This minimizes the consumption of collection plates. Set Options ☒ Continuous Fraction Collection
2	Define the chromatographic workflow by setting a desired number of process steps.

Process step configuration

Follow the actions below to configure each individual process step.

Step	Action
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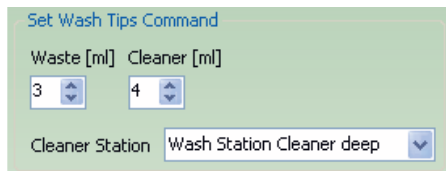
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|---|--|
| 1 | Edit a Process Step Name , for example Equilibration. |
|---|--|



- | | |
|---|---|
| 2 | Check the Process Full Plate box according to the desired procedure: |
|---|---|

- When checked: The fullPreDictor RoboColumn array will be processed.
- When not checked: The PreDictor RoboColumn array will be processed column per column.

- | | |
|---|--|
| 3 | Set parameters for tip washing. The default volume for Waste is 3 ml and for Cleaner 4 ml. The volumes need to be adapted according to the process step. |
|---|--|



- | | |
|---|---|
| 4 | Set individual chromatographic parameters. <ul style="list-style-type: none">a. Select the source of the liquid.b. Set the dispensing volume (i.e., the volume for the chromatographic step) or check the Use absolute volume box to enter an absolute volume.c. Set the required liquid class for aspiration. |
|---|---|

Step	Action
------	--------

- d. In the Te-Chrom Wizard, below **Flowrate**, set the required flow velocity [cm/h] or flow rate [μl/s].

The screenshot shows the Te-Chrom Wizard interface with the following settings:

- Set Source Liquid:** * Source Liquid is set to "Sample".
- Set Volume:** Resin volume is 3, Volume [μl] is 300 (calculated as 3 x 100). The "Use absolute volume" checkbox is unchecked.
- Liquid Handling Parameters:** Aspirate liquid class is set to "Aspirate_Atoll_Trough".
- Flowrate:** Set to 350 cm/h.

- 5 Check the **Engage Waste Tray** box according to the desired procedure:

- When checked: Depending on the waste tray positioning, select either the RoMa or liquid handling arm (LiHa).
- When not checked: The fractions will be collected in the desired collection plate.

The screenshot shows the Te-Chrom Wizard interface with the following settings:

- Choose Waste Option:** The "Engage Waste Tray" checkbox is checked.
- Select Positioning:** Te-Chrom waste tray positioning is set to "RoMa" (radio button selected).

- 6 **Choose Options** according to the desired procedure:

- Check the **Neutralization** box to perform pH adjustment.
- Check the **Data Collection** box to evaluate the collection plate in a reader device (e.g., UV detector).

Step	Action
------	--------

Choose Options

☒ Neutralization

☐ Data Collection

- 7 If the **Engage Waste Tray** box in step 5 is not checked, select the **Number of Fractions** according to the given dispensing volume for the chromatographic step.

Number of Fractions

Well	Fraction Volume [μl]
A1	50
B1	50
C1	50
D1	50

- 8 Repeat steps 1 to 7 in order to configure all chromatographic process steps.

Tip: The residence time (τ) can be calculated from either the volumetric flow rate (Q) or the flow velocity (u) using the following formulas:

$$\tau = V_c / Q$$

$$\tau = V_c / (u \times A_c)$$

where V_c is the column volume and A_c the cross-sectional area of the column.

Plate handling

i Number of plates required: 2

Add the required amount of source plates.
Then add the required amount of destination plates.

Carrier: Te-Stack right MCA96 (10/0)

Site:

Number of plates:

Select Process Steps

Plates	Process Steps
0	equilibration
2	sample, wash, elution
0	regeneration

* Source

Te-Stack right MCA96 (10/1)
Te-Stack right MCA96 (10/1)

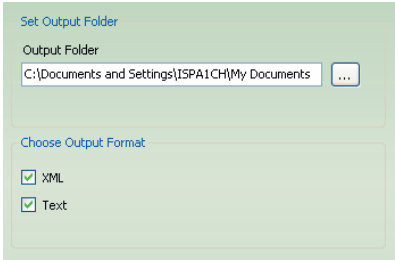
* Destination

Follow the actions below to select plate options.

Step	Action
1	Add the required number of source plates.
2	Add the required number of destination plates.

Reporting

Follow the actions below to select desired output folder and output format.

Step	Action
1	Select desired Output Folder . 
2	Select desired Output Format .

Automatic report generation

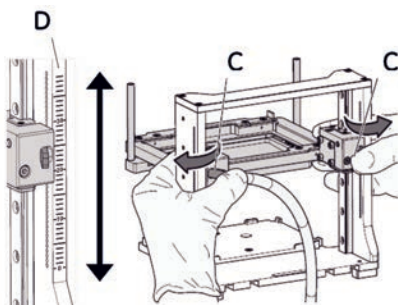
After the experimental setup the Te-Chrom Wizard automatically generates a Freedom EVOware script, representing a chromatographic method.

5.2 Detailed protocol

Follow the actions below in order to perform a complete experiment.

Step	Action
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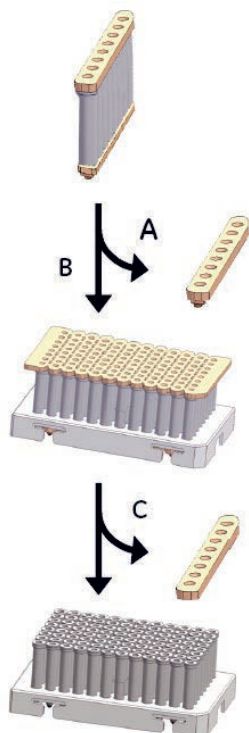
- | | |
|---|--|
| 1 | <p>Te-Chrom - Adjusting the operating height</p> <p>a. Press and hold the fastener (C) to set Te-Chrom to the required height of the Tecan Module scale (D).</p> <p>Tip: Adjust the height so that the 96- well array plate will be as close to the collection plate as possible.</p> <p>b. After the height has been manually adjusted, adjust the height parameters in the Freedom EVOware software. (See the <i>Te-Chrom Operating Manual</i> from Tecan for details).</p> |
|---|--|



- | | |
|---|--|
| 2 | <p>Remove cover seals</p> <p>a. Gently peel off the bottom seal and the row holder from the row of eight PreDicator RoboColumn units (A).</p> <p>b. Assemble the PreDicator RoboColumn units onto the 96- well array plate (B). Up to 12 rows of eight PreDicator RoboColumn units can be assembled.</p> <p>Note: Do not mix short (50 µl, 200 µl) and long (600 µl) PreDicator RoboColumn units in the same 96-well array plate.</p> |
|---|--|

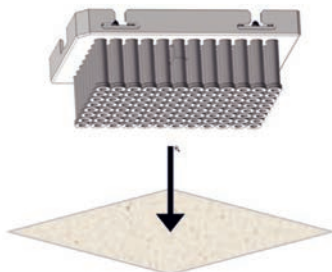
Step	Action
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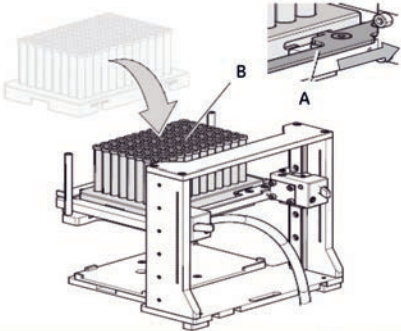
- | | |
|----|--|
| c. | Gently peel off the top seal for each row of eight PreDictor RoboColumn units (C). |
|----|--|



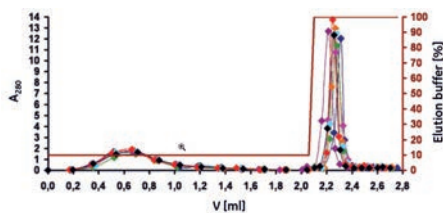
3 Remove excess storage solution

- Remove excess storage solution in the top part of the columns by blotting the 96-well array plate upside down on a soft paper tissue.



Step	Action
4	Te-Chrom - Loading the 96-well array plate - Move the locking slider (A) to the left mechanical stop. - Place the 96-well array plate (B) onto Te-Chrom. - Move the locking slider (A) to the right mechanical stop to lock the 96-well array plate in position. Note: Make sure that the PreDicator RoboColumn units are well secured into the 96-well array plate before using them. 
5	Run chromatographic method Press the green arrow in order to start 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	Data analysis - Determine the precise volume of the collected fractions. - Determine the protein concentration. - Transfer the collection plate to any location on the robotic worktable or to secondary devices by using the RoMa.
7	Data plotting Note: Make sure that blank values of the used solvents are subtracted from the UV data before plotting. Plot the response (UV data or other) against the fraction volume to create a pseudochromatogram.

Step	Action
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8	Regeneration and storage
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- For appropriate regeneration of PreDicator RoboColumn units, please refer to the recommendation for the corresponding chromatography medium. In general, regeneration of the chromatography media depends on the nature of the sample.
- For appropriate storage of PreDicator RoboColumn units, please refer to [Chapter 3 Characteristics, on page 6](#)

6 Troubleshooting

Recommended actions

See the Table below for a list of some possible errors and recommended actions.

Error	Possible cause	Action
PreDicator RoboColumn is clogged.	The sample is too viscous.	Increase dilution of the cell paste before lysis, or dilute after the lysisation.
	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 used for too many cycles.	Replace the PreDicator RoboColumn.
PreDicator RoboColumn is leaking.	The column is clogged.	See above.
	The o-ring sealing is damaged.	Check the taught X- and Y coordinates for PreDicator 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.
PreDicator RoboColumn units are lifted up from the 96-well array plate during the pull out of the robotic needles.	PreDicator RoboColumn units are not well fixed into the 96-well array plate.	Make sure that all PreDicator RoboColumn units 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 short.	Decrease the flow velocity (i.e., increase residence time) for sample loading.

7 Ordering information

For ordering information and more information about related products, accessories and related literature, see information at cytiva.com/predictor.

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7.1 Related literature

Product name	Product code
Data File: PreDicator RoboColumn	28-9886-34
Data File: PreDicator 96-well filter plates and Assist software	28-9258-39
<i>Te-Chrom Operating Manual</i>	Contact Tecan
<i>Te-Chrom Wizard Software Manual</i>	Contact Tecan

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