

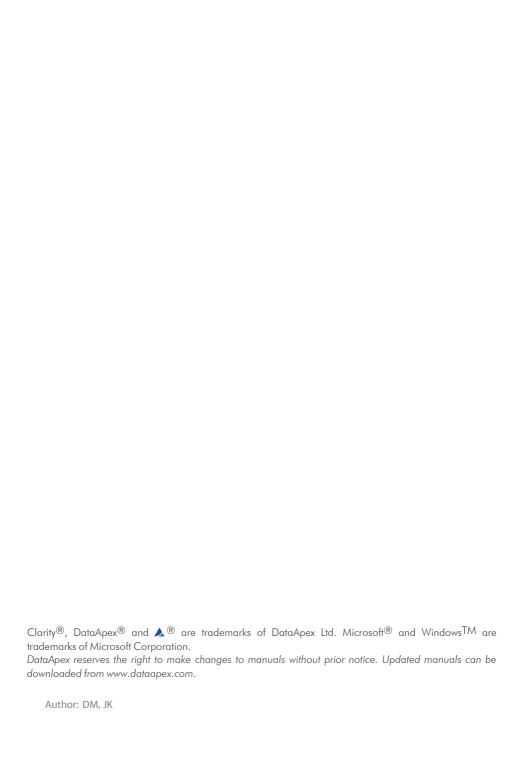
FC GP

Clarity Control Module

ENG

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Phone: +420 251 013 400 clarity@dataapex.com www.dataapex.com DataApex Ltd.
Petrzilkova 2583/13
158 00 Prague 5
Czech Republic



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To facilitate the orientation in the FC GP manual and Clarity chromatography station, different fonts are used throughout the manual. Meanings of these fonts are:

Open File (italics) describes the commands and names of fields in **Clarity**, parameters that can be entered into them or a window or dialog name.

WORK1 (capitals) indicates the name of the file and/or directory.

ACTIVE (capital italics) marks the state of the station or its part.

Chromatogram (blue underlined) marks clickable links referring to related chapters.

The bold text is sometimes also used for important parts of the text and the name of the **Clarity** station. Moreover, some sections are written in format other than normal text. These sections are formatted as follows:

Note: Notifies the reader of relevant information.

Caution: Warns the user of possibly dangerous or very important information.

Marks the problem statement or trouble question.

Description: Presents more detailed information on the problem, describes its causes,

etc.

Solution: Marks the response to the question, presents a procedure how to remove it.

1 General Purpose Fraction Collector (FC GP)

The DataApex FC GP (General Purpose Fraction Collector) control is a universal driver designed to function with Fraction Collectors controlled by Next Fraction and Collect/Waste events. The DataApex FC GP module is available for LC, GPC, LC-PDA, CE and GPC-PDA Instrument types.

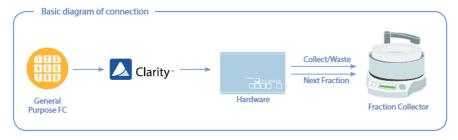


Fig. 1: General Purpose Fraction Collector

FC GP 2 Requirements

2 Requirements

- Fraction Collector controlled by Next Fraction and Collect/Waste events.
- Clarity installation with LC Control module (p/n A24) license.
- A/D converter (e.g. DataApex Colibrick).
- · Two unoccupied digital output contacts.

Note:

Although the DataApex discontinued A/D converters INT7, INT9, and Net-PAD A/D converters have 8 digital outputs each, the cables provided for them will support 4 digital outputs maximum (based on the number of channels). You may need a **SV8** or **SV9** terminal board to access the unused digital outputs.

Caution:

U-PAD and **U-PAD2** discontinued A/D converters have only 2 digital outputs, so they are generally unsuitable for the operation of the general purpose fraction collector module (as you usually need one more digital output to start the analysis).

3 Installation

- Install your A/D converter.
- Connect the Fraction Collector to the A/D card by the cable provided with the card.

3.1 Clarity Configuration

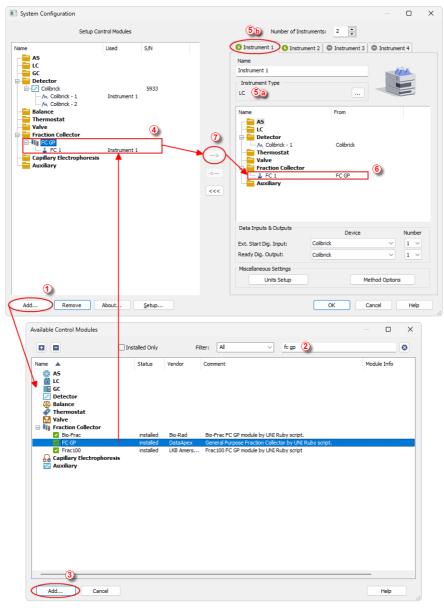


Fig. 2: System Configuration - adding the FC GP

- Start the **Clarity** station by clicking on the **L** icon on the desktop.
- Invoke the System Configuration dialog accessible from the Clarity window using the System – Configuration... command.
- Press the Add button (See Fig. 2 on pg. 4.) to invoke the Available Control Modules dialog.
- You can specify the searching filter ② to simplify the finding of the driver.
- Select the **FC GP** and press the *Add* ③ button.

The DataApex FC GP Setup dialog will appear.

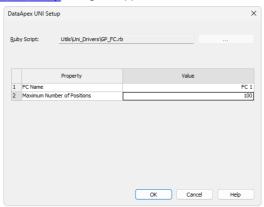


Fig. 3: DataApex FC GP Setup

• Enter the FC GP name in the FC Name field.

Note: A detailed description of this dialog can be found in the chapter "DataApex FC GP Setup" on pg. 10.

Press the OK button.

The **FC GP** item will appear in the *Setup Control Modules* list 4 of the *System Configuration* dialog.

- Change the Instrument Type (5) (a) on the desired Instrument tab (5) (b) to LC.
- Drag and drop the **FC GP** icon from the *Setup Control Modules* list 4 to the *Instrument* tab on the right 6, or use the button 7.

Note: The configuration dialog of the FC GP module (<u>DataApex FC GP Setup</u>) can be displayed any time by double-clicking on its icon or using the Setup button.

3.2 Configuring the Event Table

It is necessary to assign physical **Clarity** outputs (e.g. A/D card) to the signal coming from the virtual fraction collector module in the *Event Table* dialog. Source of the input should be set to DataApex FC GP. The *Input 1* conveys the *Collect/Waste* signal, where value *Up* means Collect and value *Down* means Waste. *Input 2* conveys the Next signal, as the value Up means Next.

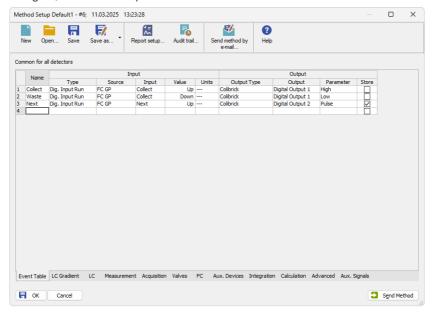
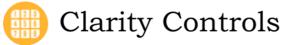


Fig. 4: Method Setup - Event Table

Relations and the data flow of the typical FC GP configuration and settings of the *Event table* are displayed in the following scheme. The settings of the *Fraction table* are individual to each analysis and have to be set in accordance with the chromatogram, signal range and other parameters.

When the conditions set in the *Fraction Table* are fulfilled, the fraction collector control module sends appropriate signals to the **Clarity** station. **Clarity** then, according to the events set in the *Event table*, sends the appropriate digital signal to the fraction collector via the A/D converter digital outputs.



FC GP Fraction Table

	Name	Start Time [min]	Stop Time [min]	Fraction Volume [mL]	Detector Signal	Signal Condition	Level Start	Level Stop	Slope Start	Slope Stop	Filter
1	Fraction 1	1.000	3.000	1.00	Colibric	Level	0.200	0.150			1
2	Fraction 2	3.000	3.200	1.00	Colibric	None					1
3	Fraction 3	3.200	3.400	1.00	Colibric	None					1
4	Fraction 4	3.400	6.000	1.00	Colibric	Level	0.200	0.150			1



Event Table

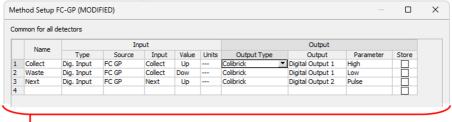




Fig. 5: Scheme of typical FC GP module operation

The FC GP can also be controlled by external signals (from the control panel of the real fraction collector or by the pushbutton). In this case it is necessary (possibly in addition to the standard event table setting) to assign real **Clarity** inputs (A/D card) to the virtual signal outgoing to the FC GP. The particular A/D card should be set as *Input Source*, while the DataApex FC GP serves as the *Output Type*.

Controlling the fraction collector using external signals is equivalent to the manual control from the *Device Monitor* with one exception - it is not possible to restart the automatic fraction collection using external signal. This process can be performed only from the *Device Monitor*.

4 Using the control module

New FC tab is created in the **Method Setup** dialog, enabling the setting of the Fraction Collector. This dialog serves for setting up the FC instrument method.

The general procedure of work will be following:

- Define the fractions based on time, signal level, slope of signal etc. in the Method Setup - FC- Fraction table.
- Set the range of vials where the fractions will be stored, and the conditions for new runs in the Method Setup - FC - Vial Numbers.
- · Start the Acquisition.
- During its run the user can see the Fractions in the Data Acquisition window,

Note: To see the fractions in the Data Acquisition window, click the right mouse button in the graph, Select Graph Properties and enable the Show Events checkbox.

- In the <u>Device Monitor</u> the user can overview and control the whole process. For example, check current fraction or its volume, force next fraction, stop collecting, etc.
- After the analysis (fraction collecting) the user can open the chromatogram, review the fractions in the graph and dedicated result table (<u>Chromatogram - FC</u> <u>Result</u>) and eventually print the report.

4.1 DataApex FC GP Setup

The *DataApex FC GP Setup* dialog serves for setting the custom names for the **FC GP** module itself and maximum number of positions.

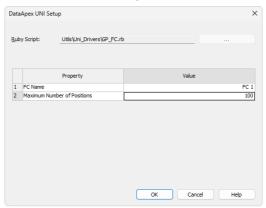


Fig. 6: DataApex FC GP Setup

FC Name

Allows to set a custom name for the **FC GP** module. Default name is *FC 1*.

Maximum Number of Positions

Allows to set a maximum number of positions. Default value is 100.

4.2 Method Setup - FC - Fraction Table

The *Method Setup - FC - Fraction Table* tab is used for setting the automatic fraction collection program of the fraction collector. To display the actions of the fraction collector in *Data Acquisition* window or *Chromatogram* window, click the right mouse button on the graph, choose *Properties...* to open the **Graph properties** dialog and check the *Show Events* option.

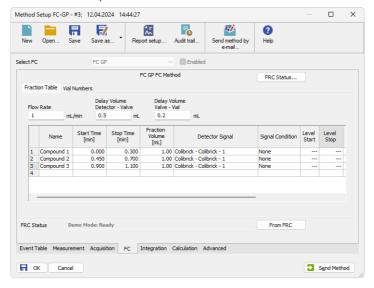


Fig. 7: FC - Fraction Table tab

Flow Rate

Specifies the flow rate of the liquid going to the fraction collector. Together with Fraction Volume parameter from the Fraction Table, it is used to prevent vial overflow

Delay Volume Detector - Valve

Defines the volume of tubing between the detector and the fraction collector's waste/collect valve.

The delayed volume can be displayed in the <u>Data Acquisition</u> window as a vertical line trailing the signal front. Choose *Properties*... to open the *Graph Properties* dialog and check the *Show Events* checkbox.

Delay Volume Valve - Vial

Defines the tubing volume between the waste/collect valve and the vial in the fraction collector tray. If the fraction collector has no waste/collect valve, leave this field empty and enter the total tubing volume from the detector and fraction collector tray in the Delay Volume Detector - Valve field.

Fraction Table

Sets up the fraction collector behavior. The table rows run in chronological order and time intervals should not overlap. If a collection is active when the interval ends, it stops immediately. Collection based on negative peaks is not supported; to collect such peaks, invert the detector signal before setting up the method.

Name

This name will be displayed in the *Special Results* table for all fractions collected in the given interval.

Start Time [min]

Beginning of the time interval where the Signal Condition will be evaluated.

Stop Time [min]

End of the time interval where the *Signal Condition* will be evaluated.

Fraction Volume

Defines the volume assigned to a single fraction. The value should not exceed the physical vial capacity to avoid overflow. When the collected volume reaches the set value, the fraction collector automatically switches to the next vial.

Detector Signal

Specifies the detector signal used to trigger fraction collection according to the Signal Condition in the Fraction Table. Each row of the table can be linked to a different detector signal. Only detector signals that are enabled for acquisition are available for use in the *Fraction Table*.

Signal Condition

Signal Condition is evaluated within the time interval defined for each row in the Fraction Table. If the condition is met multiple times during the interval, fraction collection starts each time, with the vial automatically changed after every stop. If the condition is not met at all, no collection is performed for that interval.

None - The fractions are collected for the entire time interval defined by *Start Time* and *Stop Time* regardless of the detector signal

Note:

If two Fraction Table rows follow each other directly in time (Stop Time = Start Time) and both have the Signal Condition = None, the FC does not switch to Waste between the rows. Skipping the switch to Waste is possible only if the collector still has a free vial available and the Waste During option in the Vial Change method is not selected.

Level - The fraction collection:

- starts when the detector signal increases above the Level Start
- stops when the detector signal decreases below the Level Stop

Slope - The fraction collection:

- starts when the first derivative of the detector signal increases above the Slope Start
- stops when the first derivative of the detector signal first decreases and then
 increases above the negative value of the Slope Stop. The following image

displays the course of the first derivative and demonstrates *Slope Stop* activation. Carefully read the slope values at all *End Fraction* points and observe the course of the first derivative before it reaches these points.

For accuracy, the Slope Stop condition is applied with a tolerance of 5%. The entered value is multiplied by 1.05 (e.g., an entered value of 0.100 is evaluated as 0.105).

It is strongly recommended not to use Slope Signal Condition for fraction collection based on noisy detector signal.

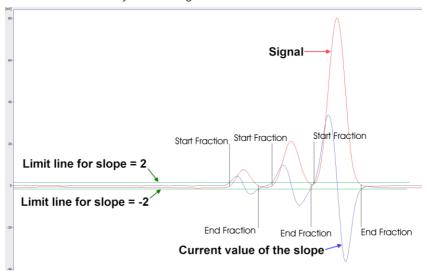


Fig. 8: First derivative course

Start L+S End L+S - The fraction collection:

- starts when both start signal conditions are fulfilled simultaneously, detector signal increases above Level Start value and also the first derivative of the detector signal increases above Slope Start value.
- stops when both stop signal conditions are fulfilled simultaneously, detector signal decreases below Level Stop value and also the first derivative of the detector signal first decreases and then increases above the negative of the Slope Stop value. If only one of the stop signal conditions is fulfilled the fraction collection will not stop until Stop Time is reached.

Start L+S End L or S - The fraction collection:

 starts when both start signal conditions are fulfilled simultaneously, detector signal increases above Level Start value and also the first derivative of the detector signal increases above Slope Start value. stops when any of the stop signal conditions is fulfilled (whatever occurs first), detector signal decreases below *Level Stop* value or the first derivative of the detector signal first decreases and then increases above the negative of the *Slope Stop* value. If none of the stop signal conditions is fulfilled the fraction collection will not stop until *Stop Time* is reached.

Start L or S End L+S - The fraction collection:

- starts when any of the start signal conditions is fulfilled (whatever occurs first), detector signal increases above Level Start value or the first derivative of the detector signal increases above Slope Start value.
- stops when both stop signal conditions are fulfilled simultaneously, detector signal decreases below Level Stop value and also the first derivative of the detector signal first decreases and then increases above the negative of the Slope Stop value. If only one of the stop signal conditions is fulfilled the fraction collection does not stop until Stop Time is reached.

Start L or S End L or S - The fraction collection:

- starts when any of the start signal conditions is fulfilled (whatever occurs first), detector signal increases above Level Start value or the first derivative of the detector signal increases above Slope Start value.
- stops when any of the stop signal conditions is fulfilled (whatever occurs
 first), detector signal decreases below Level Stop value or the first derivative
 of the detector signal first decreases and then increases above the negative
 of the Slope Stop value. If none of the stop signal conditions is fulfilled the
 fraction collection does not stop until Stop Time is reached.

Filter

Sets the data filter for the detector signal defined in the corresponding row of the *Fraction Table*. The default value of 1 means no filtering, value of X means that every X incoming data points are averaged into one point used for evaluating fraction start/stop conditions. The higher the *Filter* value, the greater the delay in responding to signal conditions, especially with detectors that have a low data rate.

Filter settings influence the value that should be set to the *Slope Start* and *Slope Stop* columns. To determine the correct trigger values for the *Slope Start* or *Slope Stop* with the *Show Slope/Level* tool in the *Chromatogram* window, the *Global Filter – Bunching* value in the *Integration Table* should match the *Filter* value set here.

The values coming from *Detector Signal* are evaluated in bunch approximately every 300 milliseconds with respect to Start and Stop values inserted in the *Fraction Table*. As a result, the positions of fraction collection start and stop events may vary by ± 300 ms between chromatograms with identical signal curves. For detectors with a high sampling rate (e.g., 50 Hz), this corresponds to a variation of up to ± 15 data points (50 Hz = 50 points per second; 300 ms \approx 15 points).

4.3 Method Setup - FC - Vial Numbers

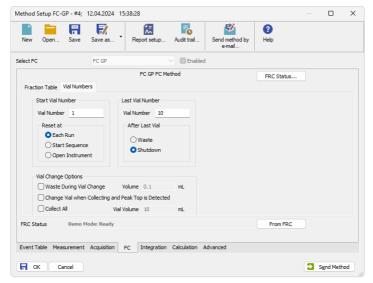


Fig. 9: FC - Vial Numbers tab

Start Vial Number

Vial Number

Sets the number of the vial where the first collected fraction will be stored. The current vial number is incremented each time when the Next signal is activated. The information about the vial number is stored in the Result Table.

Reset at

Specifies the conditions of the current vial number setting to the *Start Vial Number*. This is possibly done in three different situations:

- Each Run vial number is reset at the start of each acquisition.
- Start Sequence vial number is reset only at the start of sequence.
- Open Instrument vial number is reset only once, when the Instrument is opened.

Last Vial Number

Vial Number

Sets the number of the last available vial.

After Last Vial

Defines the action when the current vial number exceeds the Last Vial Number.

- Waste the acquisition continues, but the fraction table is ignored and everything is automatically wasted.
- Shutdown the acquisition is immediately stopped and the analysis aborted.

Vial Change Options

Waste During Vial Change

This option can be enabled to prevent spilling when the vial is changed during collecting. It temporarily stops the fraction collection before changing the vial and restarts it again when the vial is changed. The vial is changed in the middle of the wasting interval; the volume wasted during a vial change can be specified (*Volume* field). Volume units are taken from the *Instrument Method Sending* dialog.

Change Vial when Collecting and Peak Top is Detected

If enabled, a vial is automatically changed, when a peak top is detected during collecting.

Collect All

When checked, all the peaks during acquisition are collected, regardless of settings in the *Fraction Table*. FC module is then sending *Next Fraction* command only, so this option is feasible for fraction collector without a waste valve installed. Maximum amount of eluent collected in a single vial is limited by the value in the *Vial Volume* field

4.4 Data Acquisition window

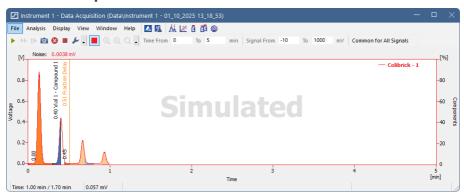


Fig. 10: Data Acquisition window

The *Data Acquisition* window can display the fractions in the graph using the background color and also the start and stop fraction events and marker of the Fraction Delay. To see the fractions and markers right click the graph, choose *Properties...* to open the *Graph Properties* dialog and check the *Show Events* checkbox.

Note:

The Fraction Delay in the picture will appear if the *Delay Volume* field in the Method Setup - FC - Fraction Table dialog is set to nonzero value.

4.5 Device Monitor

The *Device Monitor* window can be invoked by the *Monitor Device Monitor* command from the *Instrument* window or using the *Device Monitor* icon.



Fig. 11: Device Monitor

It is possible to control the fraction collection manually from the *Device Monitor*. The automatic fraction collection is paused immediately after the first manual action is executed; it can be resumed again from the *Device Monitor* window.

Vial Number

Number of the current vial 1 used for fraction collection.

Next Vial

Manually changes the vial to the next one.

Current Volume/Waste

Displays the volume collected in the vial so far ② . Text will change to *Waste*, when the fraction collector is not collecting.

Start Collecting/Stop Collecting

Manually starts the fraction collection. During the manual collection the text changes to *Stop Collecting* and the button can be used to stop the collection. As soon as the manual collection stops, text changes back to *Start Collecting* and the button can be used to start another manual collection.

Resume Method

This button is enabled only when the collection is manually controlled. It stops the manual control and resumes the automatic fraction collection according to the used method.

4.6 Digital Outputs of FC GP

The **FC GP**contains two **digital TTL outputs**, which are also designed as relay contacts. The *Digital Outputs of FC GP* dialog is accessible from the main *Clarity* window using the *System - Digital Outputs...* menu item, or appropriate icon in the main window.



Fig. 12: Digital Outputs of FC GP

Output no.

Lists the numbers of individual outputs.

Initial State

Selects the **Initial** status of the digital output when the station starts. The status is indicated in two halves: the first shows the digital output (*Output*) and the second its usability as a relay (*Relay*).

Current State

Displays and enables modification of the current status of outputs. The status is indicated in two halves: the first shows the digital output (*Output*) and the second its usability as a relay (*Relay*).

Change in these columns takes effect immediately. To change the status click the corresponding field with the inscription *HIGH* or *LOW* indicating the output value. The output status is also indicated by color, where gray corresponds to logical zero (*LOW*) and green to logical one (*HIGH*).

Descriptions

Outline description indicating the meaning of individual outputs. Default names for Output 1 and Output 2 are *Collect* and *Next*, respectively.

Note:

It is not possible to access the *Digital Outputs* dialog of the device that is configured solely on the Instrument you do not have the access to. The access to particular Instruments for different users may be set in the *User Accounts* dialog.

4.7 Chromatogram window

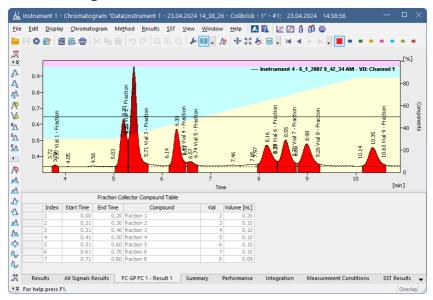


Fig. 13: Chromatogram - FC Result window

A new FC Result tab was added to the Chromatogram window. The Fraction Collector Compound Table presented on it contains the information on collected fractions.

Index

The index of the collected fraction.

Start Time

The time when the collection of the respective fraction was started. The Start Time is shown in minutes.

End Time

The time when the collection of the respective fraction ended. The End Time is shown in minutes.

Compound

Name of the fraction collected. The names used are taken from the *Fraction Table* in the *Method Setup - FC* dialog.

Vial

The number of the vial with the collected fraction.

Volume

The volume of the fraction collected. Volume units are taken from *Instrument Method Sending* dialog.

If the volume of any fraction during the analysis exceeds the fraction volume set in the *Fraction Table* of the *Method Setup - FC* dialog, the *Fraction Collector Compound Table* will contain several rows with increasing Index and Vial Numbers, but the same Compound name.

4.8 Report Setup

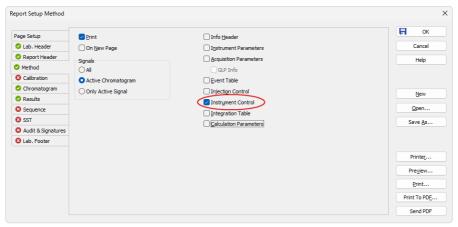


Fig. 14: Report Setup - Method dialog

Fraction Collector parameters can be displayed and printed in the report by checking the *Instrument Control* checkbox in the *Report Setup - Method* tab.

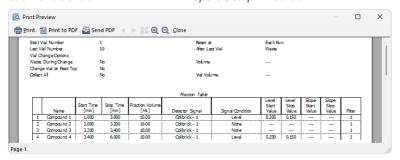


Fig. 15: Print Preview

All parameters from the *Method Setup - FC* dialog are displayed and printed, along with the data from other control modules such as GC or LC.

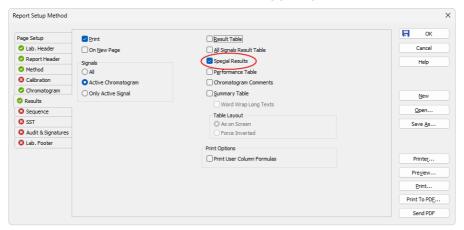


Fig. 16: Report Setup - Results Settings

It is also possible to print the Result Table generated by the FC GP module in the *Chromatogram* window by checking the *Special Results* checkbox in the *Report Setup - Results* tab.

FC GP 5 How to

5 How to

The General Purpose Fraction Collector (FC GP) can be configured to enable large variety of options to control various fraction collectors that are controlled using the Collect/Waste input (see Requirements).

The "How to" section describes certain configurations and procedures in detail to make it easier for users to set the commonly required options.

5.1 Bio-Rad BioFrac

This fraction collector offers *Advance* and *Waste/Collect* inputs, thus it can be controlled using FC GP and digital outputs of a DataApex A/D converter or any other controlled device offering auxiliary TTL and relay outputs (outputs need to be programmable from the **Clarity** Event Table). *Advance* input is offered by 15-pin D connector and *Waste/Collect* signal by 8-pin mini-DIN connector on the rear side of the FC. A cable connecting controlling device outputs to these inputs is necessary for proper using the fraction collector with **Clarity**. Such custom cable can be ordered together with **Clarity** software (in case a DataApex A/D converter is used), or manufactured separately.

In case of manufacturing the cable, the recommended connection is the following:

- Connect Advance TTL output of the controlling device to the pin nr. 1 on 15-pin D connector on BioFrac. Ground pin nr. 15 needs to be connected to the corresponding ground lead of the controlling device. For successful sending of the Advance command, the length of the pulse has to be 100 ms.
- Connect Waste/Collect relay output leads of the controlling device to the pins nr.
 2 and 8 on BioFrac mini-DIN connector. Switching the relay into "ON" position (output leads are connected) causes the diverter valve to change to collect.

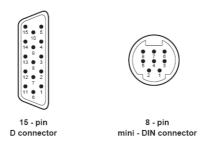


Fig. 17: BioFrac rear side connectors used for connection with controlling device.

A detailed description of the FC connectors is available in the BioFrac Instruction Manual, and the A/D converter output is described in the Colibrick manual.

FC GP 5 How to

For proper functioning it is necessary to switch the FC to the *LP/Econo* operation mode, according to the BioFrac Instruction Manual. It is also necessary to set *Rack*, *MultiRun*, *Start Tube* and *End Tube* parameters from fraction collector Main Screen.