

# **PA 800 Plus Empower<sup>™</sup> Driver**

User Guide



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This document provides instructions for using Waters Empower<sup>™</sup> Software with a PA 800 Plus System. The PA 800 Plus Empower<sup>™</sup> Driver must be installed on the computer with the Empower<sup>™</sup> Software. Refer to the *PA 800 Plus Empower<sup>™</sup> Driver Release Notes* for installation instructions.

This document includes instructions for calibrating the detectors in the PA 800 Plus System. Instructions for direct control of the PA 800 Plus System using the Empower<sup>™</sup> Software are also provided.

Note: Refer to the System Overview Guide for instructions for safe use of the system.

The Empower<sup>™</sup> Software can also be used with the CESI 8000 Plus High Performance Separation-ESI Module if an LIF, PDA, or UV detector is installed.

# **Related Documentation**

This document assumes some knowledge of the Empower<sup>™</sup> Software. For instructions on general features of the Empower<sup>™</sup> 3 (FR4) Software:

- Refer to the documentation supplied with the software.
- Click 
   on the Empower Start dialog
- Click **Help** in any of the Empower<sup>™</sup> Software programs.

For detailed instructions on using the Empower<sup>™</sup> Software for a specific capillary electrophoresis application, refer to the following application guides.

- Fast Glycan Labeling and Analysis Kit Application Guide
- Capillary Isoelectric Focusing (cIEF) Analysis Application Guide
- IgG Purity and Heterogeneity Assay Kit Analysis Application Guide

For information about the PA 800 Plus System:

- For a general introduction to the system, refer to Chapter 1 in the PA 800 Plus Pharmaceutical Analysis System Overview Guide.
- For instructions on maintaining the system, refer to the PA 800 Plus Pharmaceutical Analysis System Maintenance Guide.

# Empower<sup>™</sup> Software Terminology for 32 Karat<sup>™</sup> Software Users

Users who have used the PA 800 Plus System with the 32 Karat<sup>TM</sup> Software will need to become familiar with the Empower<sup>TM</sup> Software terms.

32 Karat <sup>™</sup> Software Term	Empower <sup>™</sup> Software Equivalent	Description
No equivalents in 32 Karat <sup>™</sup> Software	Instrument Method	A method containing system parameters required for data acquisition. Parameters are grouped as general parameters, detector parameters, and a time program.
	Processing Method	A method containing data processing parameters.
	Reporting Method	A method to create a report showing the results of the processing method.
Method	Method Set	A combination of an instrument method, a processing method, and a report method. Processing and report methods are optional.
Sequence	Sample Set Method	A list of samples and associated method sets that is sent to the PA 800 Plus System for data acquisition. Optionally, the Empower <sup>™</sup> Software can perform post-acquisition data processing and generate reports.
Report	Report	A file containing information about the results of the data acquisition. Reports can also include information about the organization generating the data. The layout and appearance of a report can be customized and saved as part of a report template.
Sample Tray	Plate	The tray or 96-well plate that holds the samples to be analyzed.

Table 1-1 Empower <sup>™</sup>	<sup>1</sup> Software Terminology for	<sup>7</sup> 32 Karat <sup>™</sup> Software Users
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32 Karat <sup>™</sup> Software Term	Empower <sup>™</sup> Software Equivalent	Description
Buffer Tray	Plate	The tray that holds the vials that contain the buffer and rinse solutions.
Controller	LAC/E module	The computer that controls the PA 800 Plus System.

Table 1-1 Empower<sup>™</sup> Software Terminology for 32 Karat<sup>™</sup> Software Users (continued)

# PA 800 Plus Empower<sup>™</sup> Driver License

To collect and analyze data with the PA 800 Plus  $Empower^{TM}$  Driver a USB license key is required. The license key should be inserted in a USB port on the  $Empower^{TM}$  Software LAC/E acquisition server.

If the license key is not present, then all controls in the **Direct Control** pane are disabled. In addition, data acquisition will not begin. If the license key is removed during data acquisition, then acquisition for the current method set finishes but no additional data acquisition will start.

The license key can be removed from one LAC/E acquisition server and inserted in a USB port on another computer if required.

This section describes how to control the PA 800 Plus System using the Direct Control pane in the  $Empower^{TM}$  Software.

There are three sections in the Direct Control pane. From top to bottom:

- Instrument status pane: Shows the status of the system. Refer to Instrument Status in the Direct Control Pane.
- Status field: Shows the status of the system or any process taking place on the system. Errors are also shown in red text in this field
- Parameter tabs and buttons: Set the parameters for the system. Depending on the type of detector, different tabs are shown. Refer to Parameters and Buttons in the Direct Control Pane.

Figure 2-1 Direct Control Pane (PDA Detector)

Control —		- Temperature		- Tray -		PDA [	Detecto	r	
Voltage	0.000 kV	Cartridge 2	25.6 °C	BI:	A1	Chan	Wv	Bw	Absorb.
Current	Au 000.0	Storage 2	25.0 °C	BO:		Ch1	214	10	0.000000
Power	0 W			Lamp	Hrs	Ch2	214	10	0.000000
Pressure -		-Coolant -	Lamp	D2:	7.00 h	Ch3	214	10	0.000000
0.0	psi ⊏>+	ОК	On	Hg:	0.06 h	Ref.	214	10	0.000000
Status Idle	•								
Voltage S	Settings   Ter	mperature   La	mp On/Off	Spec	trum   Li	amp Hou			Home
🗸 🗸 🗸	/S								Load
	Inlet Sample	Tray	48 Vials					II —	
	Inlet Buffer T	ray	36 Vials						Set
	Outlet Samp	le Tray	96 Positi	ions/N	lo Tray				Stop
	Outlet Buffer	Tray	36 Vials					II —	Stop
									Advanced
							<b>\$</b>	P/	۵00 Plus

# **Instrument Status in the Direct Control Pane**

**Note:** Pressure values can be shown in millibar (mbar) or pounds per square inch (psi), depending on a registry setting for the Empower<sup>TM</sup> Software. The default unit is millibar. To change the units, refer to the *PA 800 Plus Empower<sup>TM</sup> Driver Release Notes.* 

- Control -		Temperatu		Trave		- LIE Detector	r
Valtage	0.000 14	/ Centridae	24.0 °C	DI	A1	Changele	DELL
voitage	0.000 KV	Cantridge	24.0 C	DI.	AI	Channels	REU
Current	0.000 μ/	A Storage	25.0 °C	BO:	A1	Ch1	0.000000
Power	0.000 W			Lase	r Hrs —	Ch2	0.000000
Pressure	) ————	Coolant	Lasers -	1:	12.50 h		
0.0	)psi <b>⊏&gt;+</b>	ОК	Off	2:	0.00 h		

Label	Description						
Control	Shows the voltage, current, and power.						
Temperature	Shows the temperature of the cartridge and sample cooling system.						
Tray	Shows the location of the capillary inlet and outlet.						
LIF Detector	Shows information about the LIF detector.						
	• Channels: The channel for the data, Ch1 and Ch2.						
	• <b>RFU</b> : The relative fluorescence units of the data in this channel.						
Pressure	Shows the direction and the magnitude of the pressure or vacuum.						
	<ul> <li>➡: forward direction</li> </ul>						
	• 🗢: reverse direction						
	• +: pressure						
	• -: vacuum						
Coolant	Shows the status of the coolant, OK or Low.						

#### Figure 2-2 Instrument Status in the Direct Control Pane (LIF Detector)

Label	Description
Lasers	(LIF detector) Shows the status of the laser, On or Off.
Laser Hrs	(LIF detector) Shows the number of hours that the laser has been on.
	• 1 Hours for the integrated 488 nm laser.
	• 2 Hours for an external laser, if installed.

#### Figure 2-3 Instrument Status in the Direct Control Pane (PDA Detector)

Control -		- Temperatu	re	- Tray -			Detecto	r	
Voltage	0.000 kV	Cartridge	25.6 °C	BI:	A1	Chan	Wv	Bw	Absorb.
Current	0.000 μA	Storage	25.0 °C	BO:		Ch1	214	10	0.000000
Power	0 W			Lamp	Hrs	Ch2	214	10	0.000000
Pressure -		-Coolant -	Lamp	D2:	7.00 h	Ch3	214	10	0.000000
0.0	psi <b>⊏&gt;+</b>	ОК	On	Hg:	0.06 h	Ref.	214	10	0.000000

Label	Description							
Lamp	Shows the status of the lamp, On or Off.							
Lamp Hrs	Shows the number of hours that the lamps have been on.							
	• D2: The number of hours the deuterium lamp has been on							
	• <b>Hg</b> : The number of hours the mercury lamp has been on.							
PDA Detector	Shows information about the PDA detector.							
	Chan: The channel for the data.							
	• Wv: The wavelength for the channel, in nm.							
	• <b>Bw</b> : The bandwidth for the channel, in nm.							
	Absorb: The absorbance for the channel.							

Note: For items common to all detector types, refer to Figure 2-2.

#### Figure 2-4 Instrument Status in the Direct Control Pane (UV Detector)

Control -		- Temperatu	re	Tray		UV De	etector -	
Voltage	0.000 kV	Cartridge	25.2 °C	BI:	A1	Chan	Wv	Absorb.
Current	Αμ 000.0	Storage	25.0 °C	BO:	A1	Ch1	0	0.000000
Power	0.000 W			Lamp	Hrs			
Pressure -		-Coolant -	Lamp —	D2:	5.50 h			
0.0 p	osi <b>⊏&gt;+</b>	ОК	On	Hg:	0.00 h			F

Note: For items common to all detector	or types, refer to Figure 2-2.
--	--------------------------------

Label	Description		
Lamp	Shows the status of the lamp, On or Off.		
Lamp Hrs	Shows the number of hours that the lamps have been on.		
	• <b>D2</b> : The number of hours the deuterium lamp has been on.		
	• <b>Hg</b> : For display only. Not used for the UV detector.		
UV Detector	Shows information about the UV detector.		
	Chan: The channel for the data.		
	• Wv: The wavelength for the channel, in nm.		
	• Absorb: The absorbance for the channel.		
F	Click to view filter information.		

#### Figure 2-5 Instrument Status in the Direct Control Pane (UV Filters)

- Control	- Temperature	Tray	UV Filters	
Voltage 0.000 kV	Cartridge 25.2 °C	BI: A1	F 1: F 6:	0
Current 0.000 µA	Storage 25.0 °C	BO: A1	F 2: 200 F 7:	0
Power 0.000 W		Lamp Hrs	F3: 214 F8:	0
Pressure 0.0 psi <b>c&gt;+</b>	Coolant Lamp OK Off	D2: 5.50 h Hg: 0.00 h	F 4: 254 F 5: 280	D

Note: For items common to all detector types, refer to Figure 2-2.

Label	Description
Lamp	Refer to Figure 2-4.
Lamp Hrs	Refer to Figure 2-4.
UV Filters	<b>F<x></x></b> : Shows the wavelength of the filter in position <x>, in nm.</x>
D	Click to view detector information.

# Parameters and Buttons in the Direct Control Pane

**Note:** Pressure values can be shown in millibar (mbar) or pounds per square inch (psi), depending on a registry setting for the Empower<sup>TM</sup> Software. The default unit is millibar. To change the units, refer to the *PA 800 Plus Empower<sup>TM</sup> Driver Release Notes*.

Pre	ssure Settings Voltage Settings	Temperature   Lamp On/Off   La	Home
	Pressure (psi)	0.1	Load
	Duration (min)	0.10	
	Pressure Direction	Forward	Set
~	Tray Positions		Stop
	Inlet Vial	A1	
	Inlet Tray	Buffer	Advanced
	Outlet Vial	A1	
	Outlet Tray	Buffer	
			PA 800 Plus

#### Figure 2-6 Parameters and Buttons in the Direct Control Pane

Label	Description	
Parameter Tabs		
Pressure Settings	Set the pressure for the system.	
Voltage Settings	Set the voltage for the system.	
Temperature	Set the temperature for the capillary and sample cooler.	
Lamp On/Off	(UV or PDA detector) Turn the lamp on or off.	

Label	Description		
Laser On/Off	(LIF detector) Turn the laser on or off.		
Calibration Factors	(LIF detector) View the calibration correction factors and set the parameters for detector calibration. Refer to Calibrate the LIF Detector.		
UV Filters	(UV detector) Set the position and wavelength of filters installed in the system.		
Lamp Hours	(UV or PDA detector) After replacing the lamp, set the lamp hours to 0.		
Lamp Energy	(UV detector) Select filter in the <b>Filter</b> list and then click <b>Set</b> to view the current between the diodes in the deuterium lamp, in nA. This value decreases over time due to the lamp aging.		
Trays	View the type of sample and buffer trays in use.		
Spectrum	(PDA detector) View the spectrum of the deuterium lamp. Refer to View the Deuterium Lamp Spectrum and Intensity.		
Buttons			
• •	Click to view the next or previous tab.		
<b></b>	Click to view the help pane.		
(ja	Click to close the help pane.		
Home	Click to move the trays to the home position.		
Load	Click to move the trays to the load position.		
Set	Click to send the parameters to the PA 800 Plus System.		
	• (LIF detector) When the <b>Calibration Factors</b> tab is shown, this button changes to <b>Start</b> .		
	<ul> <li>(UV detector) When the Lamp Hours tab is shown, this button changes to Reset.</li> </ul>		
	<ul> <li>(PDA detector) When the Spectrum tab is shown, this button changes to Monitor.</li> </ul>		
Stop	Click to turn off the voltage, current, power, pressure, and cooling.		
Advanced	(PDA detector) Click to calibrate the PDA detector. Refer to Calibrate the PDA Detector.		

 In the Empower<sup>™</sup> Software Project window, click File > New Method > Instrument Method. The Select Desired Chromatography System dialog opens.

Figure 3-1 Select Desired Chromatography System Dialog

Select Desired Ch	romatography Sys	tem			Х
Please select the	chromatographic sy	stem which you	would like to use to acc	juire samples into this projec	st.
Note that you may	have access to tw	o or more system	ms with the same System	n Name on different nodes.	
System Name	System Location	Node Name	System Comments		
Instrument 2		Lace3	instruments 2 in Dual		
instruments		Lacez	CES		
1					
					_
			ОК	Cancel Help	

2. Click the system to be used and then click **OK**.

Make sure that the instrument is configured with the required detector for the application.

The Instrument Method Editor opens.

3. Click the **Detector** tab, select the detector from the **Detector Type** list, and then set the parameters. Refer to Detector Parameters for an Instrument Method.

**Note:** If it is necessary to change **Detector Type**, change it first before making any other changes to the instrument method. When the **Detector Type** changes, all parameters are set to their default values.

General Detector Time Program	
Detector Type PDA -	
Electropherogram Scan Data	Filter
Data Rate   4   T     Scan Range from   190   to	General Purpose 16-25
Electropherogram Channel Data	Relays
Data Rate 4 💌 Hz	Relay 1 Closed  Closed  Closed
Acquire Ref WI[nm] Bw[nm]	Reference Channel
Channel 1  214 10	Wavelength 400 nm
Channel 2 🔲 🔲 254 10	Bandwidth 10 nm
Channel 3 🔽 🔽 280 10	
Peak Detect.  250 120	Absorbance Signal
	Signal Direct 💌

#### **Figure 3-2 Detector Parameters**

4. Click the **General** tab and then set the parameters. Refer to General Parameters for an Instrument Method.

General Detector Time Program	
Auxiliary Data Channels	Peak Detect Parameters
□ Voltage Max: 30.0 kV	Peak Noise Multiplier 2
Current Max: 300.0 µA	Peak Filter Width 9
Power Max: 9.000 W	
Pressure	Capillary Settings
Cartridge Temperature	Capillary Total Length 60.2 cm
	Capillary Length 50.0 cm
Trigger Settings	
Wait For External Trigger	
Wait for Temperature	Cartridge 25.0 °C
Do not wait	Sample Storage 25.0 °C
- Inlet Trays	Outlet Trays
Buffer 36 vials	Buffer 36 vials
Sample 48 vials	Sample No tray

Figure 3-3 General Parameters

5. Click the **Time Program** tab and then add events to the time program. Refer to Add Events to the Time Program for an Instrument Method.

The Empower<sup>TM</sup> Software requires that the last event in the time program is an **End** event.

#### Figure 3-4 Time Program

General	Detector	Time Program							
	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary
•		Rinse Pressure	20.0 psi	2.00 min	A1	Buffer	A1	Buffer	Forward;0;0
	0.00	Separate Pre 💌	20.0 psi	2.00 min	B1	Buffer	B1	Buffer	Forward;0;0
	0.20	Autozero 💌							
	2.00	End 💌							
*		-							

- 6. Save the instrument method.
  - a. Click **File > Save** to open the Save current Instrument Method dialog.
  - b. Type a name in the **Name** field.
  - c. (Optional) Type information in the **Method Comments** field.

d. If prompted, type the Empower<sup>™</sup> Software login password for current user in the **Password** field and then click **Save**.

The instrument method is saved to the current project.

### **General Parameters for an Instrument Method**

General Detector Time Program	
Auxiliary Data Channels         Voltage       Max:       30.0       kV         ✓       Current       Max:       300.0       μA         Power       Max:       9.000       W         Pressure       Cartridge Temperature	Peak Detect Parameters         Peak Noise Multiplier         Peak Filter Width         9         Capillary Settings         Capillary Total Length         G0.2       cm         Capillary Length       50.0       cm
Trigger Settings Wait For External Trigger Wait for Temperature Do not wait	Temperature       Cartridge       Sample Storage       25.0       °C
Inlet Trays Buffer 36 vials Sample 48 vials	Outlet Trays Buffer 36 vials Sample No tray

Figure 3-5 General Parameters for an Instrument Method

#### **Create an Instrument Method**

Label	Description	
Auxilliary Data Channels	Select additional types of data to be collected: <b>Voltage</b> , <b>Current</b> , <b>Pressure</b> , and <b>Cartridge Temperature</b> .	
	For <b>Voltage</b> , <b>Current</b> , and <b>Power</b> , specify the maximum value to be applied during data collection.	
Trigger Settings	Select <b>Wait For External Trigger</b> if the method is to be triggered by an outside source or device.	
	Select an option to start the run based on temperature. Options are <b>Do not wait</b> , <b>Wait for Cartridge Temperature</b> , <b>Wait for</b> <b>Storage Temperature</b> , or <b>Wait for Cartridge and Storage</b> <b>Temperature</b> .	
Inlet Trays	Select the type of sample and buffer tray installed in the inlet positions.	
Peak Detect Parameters	Do not change the parameters in this area. They have no effect on data acquisition.	
Capillary Settings	Type the dimensions of the capillary.	
Temperature (°C)	Type the temperature for the cartridge and the sample cooler.	
Outlet Trays	Select the type of sample and buffer trays installed in the outlet positions.	

# **Detector Parameters for an Instrument Method**

General Detector Time Program	
Detector Type PDA -	
Electropherogram Scan Data	Filter
Data Rate 4 V Hz	General Purpose 16-25
Electropherogram Channel Data	Relays
Data Rate 4 V Hz	Closed  Closed
Acquire Ref William Bwilam	Reference Channel
Channel 1	Wavelength 400 nm
Channel 2 🔽 🔽 254 10	Bandwidth 10 nm
Channel 3	
Peak Detect.  250 120	
	Signal Direct 💌

Figure 3-6 Detector Parameters for a PDA Detector

Label	Description	
Detector Type	Select the type of detector.	
Electropherogram Scan Data	Set the sampling rate of the data to be collected, in Hz, and the wavelength range, in nm, to be scanned.	
	A higher rate means more data points per peak, but might lead to more noise. The optimal rate differs by analyte and should be determined during method development.	
	<b>Note:</b> The value for <b>Data Rate</b> must be between 25% to 100% of the <b>Data Rate</b> for the <b>Electropherogram Channel Data</b> .	

#### **Create an Instrument Method**

Label	Description					
Electropherogram Channel Data	Set the parameters for data collection for up to three channels. Click <b>Data Rate</b> to select the sampling rate of the data to be collected.					
	A higher rate means more data points per peak, but might lead to more noise. The optimal rate differs by analyte and should be determined during method development.					
	For each channel:					
	Select Acquire to acquire data from this channel.					
	• Select <b>Ref</b> to subtract the reference trace data from the data collected in this channel. The reference is a wavelength that is recorded and subtracted from the data in the wavelength channel.					
	• Type the <b>Wavelength</b> of the data to be collected, in nm.					
	• Type the <b>Bandwidth</b> of the data to be collected, in nm.					
Filter	Click to select the filter to be used when filtering noise in the data. Refer to About the Filter Parameter.					
Relays	For <b>Relay 1</b> and <b>Relay 2</b> , set the state to <b>Open</b> or <b>Closed</b> .					
Reference Channel	Type the wavelength and bandwidth for the reference channel, in nm.					
Absorbance Signal	Select <b>Direct</b> to show the data as received from the detector.					
	Select Indirect to invert the signal before showing the data.					

General Detector Time Program	
	Flasterkersen Changel 2
	Electropherogram Channel 2
Acquisition enabled	Acquisition enabled
Acquisition	Acquisition
Dynamic range 100 💌 RFU	Dynamic range 100 🚽 RFU
Filter	Filter
General Pumose 16-25	General Pumose 16-25
Fluorescence Signal	Fluorescence Signal
Signal Direct 💌	Signal Direct 👻
Laser/filter description - information only	Laser/filter description - information only
Excitation wavelength 488 nm	Excitation wavelength 635 nm
Emission wavelength 520 nm	Emission wavelength 675 nm
Data rate	Relays
Both channels 2 V Hz	Relay 1 Relay 2 Closed

#### Figure 3-7 Detector Parameters for an LIF Detector

Label	Description			
Detector Type	Select the type of detector.			
Acquisition enabled	enabled Select to enable data acquisition for the channel. Data can be acquired from one or both channels.			
Acquisition	Select the upper limit of the data to be collected, in RFU. If the fluorescence signal is above this limit, then peaks might be truncated.			
Filter	Select the filter to be used when filtering noise in the data. Refer to About the Filter Parameter.			
Fluorescence Signal	Select <b>Direct</b> to display the data as received from the detector.			
	Select Indirect to invert the signal before displaying the data.			

#### **Create an Instrument Method**

Label	Description
Laser/filter description - information only	Type the values for the excitation and emission wavelengths, in nm. These values are stored with the method but are not used for acquisition.
	The excitation and emission wavelengths used for data acquisition are determined by the laser wavelength and the emission filter installed in the LIF detector.
Data rate	For both channels, set the sampling rate for the LIF data to be collected, in Hz.
	A higher rate means more data points per peak, but might lead to more noise. The optimal rate differs by analyte and should be determined during method development.
Relays	For <b>Relay 1</b> and <b>Relay 2</b> , set the state to <b>Open</b> or <b>Closed</b> .

#### Figure 3-8 Detector Parameters for a UV Detector

General Detector Time Program	
Detector Type UV -	
	Filter General Purpose 16-25
Electropherogram Channel Data	Relays Relay 2
Data Rate 4 v Hz Wavelength 210 v nm	Closed  Closed
	Absorbance Signal

Label	Description	
Detector Type	Select the type of detector.	
Electropherogram Channel Data	Type the <b>Data Rate</b> , in Hz, and the <b>Wavelength</b> , in nm, for data collection.	
	A higher rate means more data points per peak, but might lead to more noise. The optimal rate differs by analyte and should be determined during method development.	
Filter	Select the filter to be used when filtering noise in the data. Refer to About the Filter Parameter.	
Relays	For <b>Relay 1</b> and <b>Relay 2</b> , set the state to <b>Open</b> or <b>Closed</b> .	
Absorbance Signal	Select <b>Direct</b> to display the data as received from the detector.	
	Select Indirect to invert the signal before displaying the data.	

#### **About the Filter Parameter**

The following types of noise filters are available. For each type of filter, a peak width can be specified. The types of filter are:

- **General Purpose**: This is the normal noise filter. It provides a high degree of smoothing with limited or minimal peak distortion and loss of resolution.
- **Max Sensitivity**: This filter reduces baseline noise. It maximizes the signal-to-noise ratio, but might cause broadening or flattening of peaks. Use this for experiments where peaks are resolved and detection limits or quantitative accuracy is most important.
- **Max Resolution**: This filter preserves peak shape, but reduces baseline noise less than the other filter options.

The peak width is the expected peak width at the base of a peak. The ranges are:

- **None**: No filtering is performed.
- <16 points: The noise filter uses the smallest number of points, which means less smoothing and more noise.
- 16 25 points: The noise filter uses an intermediate number of points.
- >25 points: The noise filter uses the largest number of points, which means more smoothing and less noise.

# Add Events to the Time Program for an Instrument Method

The time program is a table of events in an instrument method. The events are executed in order, top to bottom.

- 1. Open an instrument method and then click the **Time Program** tab.
- 2. Click the **Event** cell and then select an event. Refer to Table A-1.

General	Detector T	ime Program			
	Time (min)	Event		Value	Duration
<b>₽</b> ₩			•		
		Autozero	٨		
		Capillary Temperature End			
		Inject Pressure Inject Pressure Capillary Fill			
		Inject Vacuum Inject Voltage			
		Lasers Off	۷		

#### Figure 3-9 Event List in the Time Program Tab

Fields for the event parameters appear in the pane below the table.

3. As required, type values for the parameters in the fields to the right. Refer to Table A-2.

#### Figure 3-10 Edit Event Parameters in the Time Program Tab

Pressure (psi)	25.0
Duration (s)	100.0
Pressure Direction	Forward
Tray Positions	
Inlet Vial	A1
Inlet Tray	Buffer
Outlet Vial	A1
Outlet Tray	Buffer
Increment Every Runs[]	
Inlet	0
Outlet	0
Comments	
	Pressure (psi) Duration (s) Pressure Direction Tray Positions Inlet Vial Inlet Tray Outlet Vial Outlet Tray Increment Every Runs[] Inlet Outlet Comments

4. (Optional) To show the valid ranges for the parameters, click 🛸.

Click ն to hide the help.

5. As required, right-click a row header and select **Insert Row** to insert a row in the time program. The new row appears below the selected row.

6. As required, right-click a row header and select **Remove Row** to delete the selected row.

- 7. If this time program includes any of the Separate events such as **Separate Pressure**, **Separate Current** and so on, then add the **End** event as the last event in the time program.
- 8. Save the instrument method.
  - a. Click **File > Save** to open the Save current Instrument Method dialog.
  - b. Type a name in the **Name** field.
  - c. (Optional) Type information in the Method Comments field.
  - d. If prompted, type the Empower<sup>™</sup> Software login password for current user in the **Password** field and then click **Save**.

The instrument method is saved to the current project.

# Define the Buffer and Sample Trays

In the Empower<sup>TM</sup> Software, the sample and buffer trays in the PA 800 Plus System are referred to as "plates". Plates must be defined in the Empower<sup>TM</sup> Software. To simplify this process, SCIEX provides text files with the required information that can be imported.

**Note:** The plates should have been defined when the Empower<sup>™</sup> Software was installed. If the list of plates in the Plate Types Name table includes PA 800 Plus Sample Tray, PA 800 Plus Buffer Tray, and PA 800 Plus 96 Well Sample Tray then the plates have already been defined. The procedure is included here for reference.

- 1. Insert the PA 800 Plus Empower<sup>™</sup> Driver DVD in the DVD drive.
- 2. In the Empower<sup>™</sup> Software Start dialog, click **Configure the System**.

The Configuration Manager window opens.

3. Click **Plate Types** to show the plates that are already defined.

<b>Figure</b>	4-1	Plate	Types	in the	Configuration	Manager	Window
<u> </u>			~ .		<u> </u>		

📲 EMPOWER3FR4 as Lisa/Guest - Configuration Manager — 🗆 🗙							×			
File Edit View Records Tools Help										
<b>244</b> 3 2 × 3 6 6	9		Filter By:	)efault		•	Edit Vie <u>w</u>	<u>U</u> pd	ate	
Empower 3 Configuration	E	Plate Type Name								^
Projects	1	24 uCfuge tube, 1.5mL								
Systems	2	24 vial (open access), 2mL								
	3	384 well, 80uL								
🗐 🖳 eCord	4	48 uCfuge tube, 0.5mL								
🖉 Users	5	48 vial holder, 2mL								
Ser Groups	6	6x6								
Plate Types	7	6x8								
System Audit Trail	8	96 well (tall), 1mL								
System Audit Trail	9	96 well (tall), 2mL								
	10	96 well (tall), 350uL								
	11	96 well w/700uL insert								
	12	96 well, 300 uL								
	13	ANSI-24Tube1.5mLHolder								
	14	ANSI-24Vial4mLHolder								~
, For Help, press F1		1						32 To	otal	/

- 4. Create the plate for the buffer tray.
  - a. Right-click in the table and then select **Import from Text**.
  - b. Click **Browse** and then navigate to the PA800Plus Buffer Tray.txt file on the PA 800 Plus Empower<sup>™</sup> Driver DVD.

**Note:** If the DVD is not available, then a copy of the file is included in this document. Copy the contents and then paste it in a text file. Refer to Plate Definition Files.

c. Type PA 800 Plus Buffer Tray in the New Plate Type Name field and then click OK.

#### Figure 4-2 Import Plate Type From Text File Dialog

Import Plate Type From Text File	Х						
Waters can import a plate type definition from a text file. Browse to or type in the path and name of the plate type file, then enter the desired name of the new plate type definition.							
Plate Type Definition Import File: Browse							
D:\PA800Plus Buffer Tray	_						
New Plate Type Name:							
PA 800 Plus Buffer Tray							
OK Cancel							

The buffer tray is added to the list in the Configuration Manager window.

- 5. Repeat step 4 to create the sample trays.
  - For the 48-vial sample tray, select the PA800Plus Sample Tray.txt file and then name the plate PA 800 Plus Sample Tray.
  - For the 96-well sample tray, select the PA800Plus 96 Well Sample Tray.txt file and then name the plate PA 800 Plus 96 Well Sample Tray.

As for the buffer tray, if the plate definition file is not available, a copy is available in this document. Refer to Plate Definition Files.

**Note:** The plate definition file for the 96-well sample plate is for a standard SCIEX 96-well plate (PN 609844). To use a 96-well plate from another manufacturer, click **File > New > Plate Type** in the **Configuration Manager** window and then define the plate manually.

- 6. If the Beckman Coulter PACE MDQ Control for Waters Empower<sup>™</sup> Software Driver was previously installed, then delete any plates that were created for use with the driver. Right-click the row number for the plate and then select **Delete**.
- 7. (Optional) To view detailed information about a plate, right-click the row number for the plate and then select **Properties**.
- 8. (Optional) To delete a plate, right-click the row number for the plate and then select **Delete**. Only plates added by a user can be deleted. Pre-defined plates cannot be deleted.
- 9. Click File > Exit close the Configuration Manager window.

# **System Maintenance**

This section provides instructions for changing the UV lamp and calibrating the PDA and LIF detectors using the Empower<sup>™</sup> Software.

Listed below are additional maintenance procedures for the PA 800 Plus System. For instructions, refer to the PA 800 Plus Pharmaceutical Analysis System Maintenance Guide.

- Installing a UV or PDA Detector
- Installing the UV Detector Wavelength Filters
- Installing a LIF Detector
- Rebuild a Capillary Cartridge
- Filling Vials and Installing Vial Caps
- Cleaning the Interface Block and Ejectors
- Replace the Electrodes
- Refilling the Coolant
- Cleaning the Fiber Optics
- Cleaning the LIF Detector
- Replacing the Quad Rings
- Replace the Fuses

# **Change the Detector**

- 1. In the Empower<sup>™</sup> Software, close the Run Samples window.
- 2. In the Empower<sup>™</sup> Software Start dialog, click **Configure the System** to open the Configuration Manager window.
- 3. Click **Node** in the Empower Configuration tree control to show the available nodes.
- 4. Click the row number corresponding to the appropriate node, and then right-click **Bring Offline**.

If the system is not in use, that is if no users are connected to it or no samples are being acquired, then the software takes the system offline. If the system is in use, a message indicates that the system is in use.

5. Close any open programs and then restart the LAC/E module.

6. Change the detector. Refer to the PA 800 Plus Pharmaceutical Analysis System Maintenance Guide.

For a UV detector, make note of the positions any filters installed in the UV source optics assembly.

- 7. In the Configuration Manager window, click the row number corresponding to the appropriate node, and then right-click **Bring Online**.
- 8. Click **OK** to dismiss the message.
- 9. Do one of the following:
  - For a PDA or LIF detector, calibrate the detector. Refer to Calibrate the PDA Detector and Calibrate the LIF Detector.
  - For a UV detector, set the filter information. Refer to step 10.

10. (UV detectors only) Set the filter information.

- a. In the Direct Control pane, click <sup>[]</sup> and then click the **UV Filters** tab.
- b. For each position in the detector where there is a filter, type the wavelength of the filter.The default values are shown in the following table.

#### Table 5-1 Default Filter Wavelengths for the UV Detector

Position	Wavelength
Filter Position 2	200
Filter Position 3	214
Filter Position 4	254
Filter Position 5	280

c. Click Set.

### **View the Deuterium Lamp Spectrum and Intensity**

Use this procedure to view the raw counts from the deuterium lamp as seen by the detector. If the signal is low, this procedure can determine if the UV light intensity is low due to a problem with the lamp.

The spectrum is a better indicator of lamp life than the Lamp Hours value.

#### **Required Materials**

- PDA detector
- OPCAL cartridge (PN 144660)
- 1. Install the PDA detector. Refer to Change the Detector and the PA 800 Plus Pharmaceutical Analysis System Maintenance Guide.
- 2. In the **Direct Control** pane, click the **Lamp On/Off** tab.
- 3. Click **On** and then **Set** to turn the lamp on.
- 4. Click the Spectrum tab, select 32 in the Scan Rate list, and then click Monitor.

Figure 5-1 Spectrum Tab

Temperature   Lamp On/Off Spectrum   Lamp Hours   Trays	Home
Scan Rate: 32  Diode: Count: 0	Load
	Monitor
	Stop
	Advanced
	PA 800 Plus

When the data is collected, the spectrum is shown.

#### Figure 5-2 Spectrum Tab with Acceptable Spectrum



- 5. Inspect the spectrum and the value in the **Counts** field.
  - If the value is above 5000 and the plot is not flat on top, then the lamp is working correctly.

- If the value is less than 5000, then go to step 6.
- If the plot is flat on top, then the signal is saturated. Select **64** in the **Scan Rate** list, and then click **Monitor**.

If the plot is still flat, select **128** in the **Scan Rate** list, and then click **Monitor**.



# Figure 5-3 Spectrum Tab with Saturated Spectrum

- 6. Inspect the cartridge for the following, select **32** in the **Scan Rate** list, and then click **Monitor**.
  - Make sure that the aperture is clean.
  - Make sure that the capillary is clean and not broken.
  - Make sure that the aperture is centered on the capillary window.
  - Make sure that the fiber optic cable is clean and not broken. Clean or replace as needed.

If the value in the **Counts** field is still below 5000 at 32 Hz, then go to step 7.

7. Install the OPCAL cartridge, select 32 in the Scan Rate list, and then click Monitor.

If the value in the **Counts** field is below 10 000, then the lamp might have reached the end of its useful life or it is defective and should be replaced. Refer to Change the Deuterium Lamp.

### **Change the Deuterium Lamp**

The deuterium lamp is used by the UV detector and the PDA detector. If the baseline is excessively noisy or the lamp will not illuminate, the lamp may need replacement.

#### **Required Materials**

- Deuterium lamp
- 7/64 in. hex key
- Powder-free gloves



WARNING! Hot Surface Hazard. Before replacing a lamp, turn off the power and allow sufficient time for the lamp to cool thoroughly. A hot lamp will cause burns.

1. In the Direct Control pane, click Load.

The trays move to the load position.

- 2. Lift the cartridge cover door.
- 3. Turn the system power off and allow enough time for the lamp to cool.
- 4. Loosen the two thumb-screws on the clamp bar and then lift the bar.
- 5. Remove the capillary cartridge from the interface block.
- 6. To remove the UV optics source assembly, loosen the two thumb screws, pull the assembly forward, and then put it on a clean work surface. Refer to Figure 5-4.

#### Figure 5-4 UV Optics Source Assembly



ltem	Description
1	Access door latch
2	Access door
3	Thumbscrews (one on each side)

7. Open the UV lamp access cover on the back of the UV optics source assembly and then disconnect the lamp power plug. Refer to Figure 5-5.

#### Figure 5-5 Deuterium Lamp Assembly



ltem	Description
1	Power plug

- 8. Remove the two 7/64 in. hex screws that secure the UV lamp and then remove the lamp from the lamp housing.
- 9. Install the new UV lamp by aligning the flange guide notch in the lamp with the housing guide pin.

CAUTION: Potential Wrong Result. Make sure that an orange O-ring is installed on the lamp flange before installing the lamp. A missing O-ring decreases lamp performance.

CAUTION: Potential System Damage. Use powder-free gloves to handle the UV lamp. Fingerprints, under the high temperatures and strong UV intensity that come from operating the UV lamp, form corrosive compounds that etch the surface of the UV lamp and can cause it to break when it is turned on. When handling the UV lamp, keep the UV optical window dry and protect it from abrasion.

- 10. Install the two hex screws and then tighten them until they are snug.
- 11. Connect the lamp power plug and then close the UV lamp access cover.
- 12. Put the UV optics source assembly in the mounting location, align the two upper guide pins, and then tighten the two thumb screws.
- 13. Install the capillary cartridge in the interface block.
- 14. Lower the clamp bar and then tighten the two thumb screws.
- 15. Close the cartridge cover door.
- 16. Turn on the power.
- 17. Reset the lamp hours in the Empower<sup>™</sup> Software.
  - a. Start the Empower<sup>™</sup> Software.
  - b. In the Direct Control pane, click Lamp Hours and then click Reset.

### **Calibrate the PDA Detector**

**Note:** To make sure that analysis results are consistent over time, we strongly recommend calibrating the detector each time it is installed in the PA 800 Plus System. Also calibrate the detector after replacing the capillary in the cartridge or installing a different cartridge.

1. Turn off the PA 800 Plus System and then install the PDA detector.

Refer to the PA 800 Plus Pharmaceutical Analysis System Maintenance Guide.

- 2. Turn on the PA 800 Plus System and then allow the lamp to warm up for at least 30 minutes.
- 3. Open the Empower<sup>™</sup> Software, and then click **Run Samples**.

The Direct Control pane is visible in the Run Samples window.

**Note:** If the Direct Control pane is not visible, click **View > Control Panels > SCIEX CE**.

- Control	Temperature		Trav —			etector		
Voltage 0.000	kV Cartridge 2	25.6 °C	BI:	A1	Chan	Wv	Bw	Absorb.
Current 0.000	uA Storage 2	25.0 °C	BO		Ch1	214	10	0 000000
Power 0	W		Lamp H	rs —	Ch2	214	10	0.000000
- Proceuro		1.2mp [	דיגר	00 h	Ch2	214	10	0.000000
nessule			JZ. 7	.001	Clis	214	10	0.000000
U.U psi 🖵	+ UK	On F	Hg: ()	.06 n	Ref.	214	10	0.000000
Status Idle								
Voltage Settings	Temperature La	mp On/Off	Spectru	um   La	amp Hou	• •		Home
✓ Trays								Lord
Inlet Sa	mple Tray	48 Vials						LUGU
Inlet Bu	ffer Tray	36 Vials						Set
Outlet	Sample Tray	96 Positio	ons/No	Tray			-	Stop
Outlet 8	Buffer Tray	36 Vials						otop
								dvanced
						<b>&gt;</b>	PA	A 800 Plus

Figure 5-6 Direct Control Pane for PDA Detector

4. In the Direct Control pane, click Advanced.

The window updates to show additional parameters.

#### Figure 5-7 PDA Detector Calibration Parameters

Electropherogram Channel Data			Absorbance Signal	
	Ref	WI [nm]	Bw [nm]	Direct
Channel 1		214	10	Filter
Channel 2		254	10	General Purpose 16-25
Channel 3		280	10	Shutter
Peak Detect.		250	120	Closed
Reference Cha	innel -			
Wavelength			400	nm Apply
Danduidth			10	Cancel
DandWidth				Calibrate
Status				

5. Click **Calibrate**. Do not make any changes to the parameters.

The calibration begins. When the calibration is finished, the status field shows "87: PDA Wavelength calibration successful!", where *87* is the message code.

6. If the calibration is not successful, remove the cartridge and detector, install them again, and then calibrate.

If the calibration fails a second time, then repeat this step.

7. If the calibration fails a third time, then contact SCIEX Technical Support.

# Calibrate the LIF Detector

**Note:** To make sure that analysis results are consistent over time, we strongly recommend calibrating the detector each time it is installed in the PA 800 Plus System. Also calibrate the detector after replacing the capillary in the cartridge or installing a different cartridge.

Calibrate the LIF detector to normalize the reported values for fluorescence relative to a standard.

#### **Required Materials**

- LIF Performance Test Mix (PN 726022)
- Depending on the capillary, one of the following:
  - For bare a fused-silica capillary: Capillary Performance Run Buffer A (PN 338426)
  - For an N-CHO coated capillary: Double-deionized (DDI) water (MS-grade water filtered through a 0.2 μm filter and with resistance above 18 MΩ)
- 1. After installing the LIF detector, turn on the PA 800 Plus System and then turn on the solid state laser.
- 2. Prepare the vials for the calibration.
  - a. For a bare fused-silica capillary, dilute 100  $\mu$ L of LIF Performance Test Mix with an equal volume of Run Buffer A and then put the micro vial in a universal vial.
  - b. For an N-CHO coated capillary, add 100  $\mu$ L of LIF Performance Test Mix to a micro vial and then put it in a universal vial.
- 3. Open the Empower<sup>™</sup> Software, click **Run Samples**, and if required, log in.



Figure 5-8 Empower<sup>™</sup> Software Pro Interface Window

4. In the Run Samples dialog, click the folder for the project of interest on the left, click the system that has the LIF detector installed in the list on the right, and then click **OK**.

#### Figure 5-9 Run Samples Dialog

Run Samples		×
Project in which to acquire data:	<ul> <li>Use 'Run Samples' to run new samples at your Workstation.</li> <li>Select the desired project and system from the displayed lists.</li> </ul>	Chromatographic Systems CESI 8000 Plus @ Lace2 PA800Plus NonRohs @ Lace1 PA800Plus Rohs @ Lace1
CLEF_UV_ANALTSIS  CLEF_UV_ANALTSIS  CLEF_UV  CD Driver 1.3 Sprint 8  CLEF_UV  CD ECE_CLEF_UV  CD ECE_SDS_LIF  CD ECE_SDS_PDA  CD IgG10232019  CD LIF DETECTOR CALIBRATION  CD Merv Testing	When in the Hun Samples Window, use the system control panel to equilibrate your system, or use the Sample Set Wizard to lead you through the process of creating a Sample Set to be run on the system.	Use QuickStart Use Walk-Up

- 5. In the **Direct Control** pane, click **Load** and then put the vials in the following positions in the buffer tray.
  - Inlet buffer tray position A1: 1.5 mL Run Buffer A (for a bare fused-silica capillary) or DDI water (for an N-CHO coated capillary)
  - Inlet buffer tray position B1: 200 µL diluted LIF Performance Test Mix
  - Outlet buffer tray position A1: 1.5 mL DDI water
- 6. Set the parameters and then start the calibration.
  - a. In the **Direct Control** pane, click the **Calibration Factors** tab and then click **Auto**.

#### Figure 5-10 Calibration Factors Tab in Direct Control Pane

Voltage Settings Temper	ature   Lasers On/Off	Calibration Factors	Home
Please enter the followin	g calibration paramet	ers:	Load
Detector channel:	© 1 ○ 2		Start
Target RFU value:	1	RFU	Stop
Internal diameter:	30.0	μm	
Total length:	55.0	cm	C Manual
			PA 800 Plus

b. Click the **Detector channel** to be calibrated.

c. Type the **Target RFU value**. Refer to Table 5-2.

#### Table 5-2 Calibration Parameters by Capillary

Type of Capillary	Internal diameter (μm)	Total length (cm)	Target RFU (RFU)
Bare fused-silica	50	User-specified	15
Bare fused-silica	75	User-specified	35
N-CHO coated	50	User-specified	7

- d. Type the values for the Internal diameter and Total length of the capillary.
- e. Click **Start** and then click **Yes** in the dialog that appears.

#### Figure 5-11 Vial positions Dialog



The calibration begins and takes about 9 minutes to complete. The "Calibration Complete!" message appears.

If a message stating "No step change detected" appears, then either the capillary is plugged and the calibration solution is not flowing though it past the detector or the detector cannot detect the solution. Refer to the "No Step Change Detected" section in the *System Maintenance Guide* for troubleshooting procedures.

7. Inspect the CCF value.

#### Figure 5-12 Calibration Factors Tab After Calibration

Voltage Settings   Temperature   Lasers On/Off	Calibration Factors	Home
Calibration Complete!		Load
Detector channel:	1.21	Accept
	1.21	Cancel
	•	C Manual ← Auto PA 800 Plus

• If the CCF is between 0.1 and 10, it is acceptable. Click Accept.

**Note:** If the samples will be labeled with a dye other than fluorescein, then we recommend running a standard to make sure that the system performance is acceptable.

- If the CCF value is less than 0.1 or greater than 10, then it is outside the acceptable range. Click **Cancel** and then go to step 8.
- 8. Check the following and then repeat the calibration.
  - Make sure that the capillary dimensions in the Calibration Factors tab are correct.
  - Make sure that the correct bandpass filter is installed in the detector.
  - Fill clean vials with freshly prepared reagents, cover with clean caps, and then replace the vials in the tray.

If the CCF value is still less than 0.1 or greater than 10, then there might be a problem with the laser or the light path. Contact SCIEX Technical Support at sciex.com/request-support.

# Troubleshooting

Symptom	Possible Cause	Corrective Action
"Instrument Failure" or "System Error" messages in the Empower <sup>™</sup> Software Message Center window.	<ol> <li>The wrong version of the GPIB driver is installed.</li> <li>The wrong version of the .NET Language Runtime is installed.</li> </ol>	<ol> <li>If the National Instruments GPIB driver Version 19.0 is not installed, then install it.</li> <li>If I-488.2 .NET Language Runtime 17.0.1 for .NET Framework 4.5 is not installed, then install it.</li> </ol>
After changing the detector, "Instrument Failure" or "System Error" messages in the Empower <sup>™</sup> Software Message Center window.	After installing the new detector, the firmware settings were not downloaded from the PA 800 Plus System to the LAC/E module or the Instrument Server does not have the new settings.	Restart the PA 800 Plus System and then restart the LAC/E module or the computer that is physically connected to the instrument.
The results for the assay are very different than in shown in the <i>Application Guide</i> .	The parameters in the instrument method are not correct.	<ul> <li>Inspect the instrument method and make sure that:</li> <li>The pressure is applied to the correct side of the capillary or both. Refer to the appropriate <i>Application Guide</i>.</li> <li>The pressure values are correct for the units used by the software, either millibar or psi. Refer to the <i>PA 800 Plus Empower<sup>™</sup> Driver Release Notes</i> for instructions on changing the pressure units used in the software.</li> </ul>

Symptom	Possible Cause	Corrective Action
Results of some data processing calculations are very different than similar calculations in the 32 Karat <sup>™</sup> Software.	Some of the capillary electrophoresis-related calculations in the Empower <sup>™</sup> Software are not optimized for SCIEX systems.	Create a custom calculation for CE-specific attributes such as velocity corrected area (VCA).
There are pressure or movement errors when the vials should be incremented during a run.	The Sample Set method is not correct.	Make sure that the vial increment number agrees with the number of lines in the sample set method and matches the number of runs in the sample set method.
"Scan or Channel Data Overflow" error messages during data acquisition.	Too much data is being collected due to more than one PA 800 Plus System connected to the LAC/E module.	Do not perform data acquisition on both systems at the same time or connect each system to a separate LAC/E module.

# **Time Program Events**

This section provides a list of events and the associated parameters that can be added to a time program in an instrument method. Refer to Table A-1.

For details of the parameters, refer to Table A-2.

**Note:** The **Comment** parameter is omitted from the following table but it is available for every event.

Event	Description	Parameters
Auto Zero	Zero the detector output.	At Time (min)
Capillary Temperature	Set the capillary temperature.	<ul><li>Temperature (°C)</li><li>At Time (min)</li></ul>
End	Indicate the end of the method. Only one <b>End</b> event is allowed in a method and it must be the last event in the time program.	At Time (min)
Inject Pressure	Inject the sample using pressure.	<ul> <li>Pressure (psi or mbar)</li> <li>Duration (s)</li> <li>Pressure Direction</li> <li>Tray Positions</li> <li>Increment Every Runs</li> </ul>
Inject Pressure Capillary Fill	Inject the sample using pressure. This event allows for a higher pressure and a longer duration than the <b>Inject Pressure</b> event. Use this event to completely fill the capillary with sample.	<ul> <li>Pressure (psi or mbar)</li> <li>Duration (s)</li> <li>Pressure Direction</li> <li>Tray Positions</li> <li>Increment Every Runs</li> </ul>

#### **Table A-1 Time Program Events**

Event	Description	Parameters
Inject Vacuum	Inject the sample using vacuum.	• Vacuum (psi or mbar)
		Duration (s)
		Pressure Direction
		Tray Positions
		Increment Every Runs
Inject Voltage	Inject the sample using voltage.	Voltage (kV)
		Polarity
		Duration (s)
		Tray Positions
		Increment Every Runs
Lamp Off	Turn off the lamp at the specified time.	At Time (min)
Lamp On	Turn on the lamp at the specified time.	At Time (min)
Lasers Off	(LIF detector) Turn off the lasers at the specified time.	At Time (min)
Lasers On	(LIF detector) Turn on the lasers at the specified time.	At Time (min)
Relay On	Turn on the specified relays at the specified	Relay 1
	time.	Relay 2
		At Time (min)
Rinse Pressure	Add a rinse event that uses pressure.	• Pressure (psi or mbar)
		Duration (min)
		Pressure Direction
		Tray Positions
		Increment Every Runs
		At Time (min)
	1	

Table A-1 Time Program Events (continued)

Event	Description	Parameters
Rinse Vacuum	Add a rinse event that uses vacuum.	<ul> <li>Vacuum (psi or mbar)</li> <li>Duration (min)</li> <li>Pressure Direction</li> <li>Tray Positions</li> <li>Increment Every Runs</li> <li>At Time (min)</li> </ul>
Sample Storage Temperature	Set the temperature of the sample cooler.	<ul><li>Temperature (°C)</li><li>At Time (min)</li></ul>
Separate Current	Separate the sample using current.	<ul> <li>Current (µA)</li> <li>Duration (min)</li> <li>Ramp Time (min)</li> <li>Tray Positions</li> <li>Increment Every Runs</li> <li>At Time (min)</li> </ul>
Separate Current Pressure	Separate the sample using current and pressure.	<ul> <li>Current (µA)</li> <li>Duration (min)</li> <li>Ramp Time (min)</li> <li>Pressure (psi or mbar)</li> <li>Pressure Direction</li> <li>Tray Positions</li> <li>Increment Every Runs</li> <li>At Time (min)</li> </ul>

Table A-1 Time Program Events (continued)

Event	Description	Parameters
Separate Current	Separate the sample using current and	<ul> <li>Current (µA)</li> </ul>
Vacuum	vacuum.	Duration (min)
		Ramp Time (min)
		• Vacuum (psi or mbar)
		Pressure Direction
		Tray Positions
		Increment Every Runs
		At Time (min)
Separate Power	Separate the sample using power.	Power (W)
		Duration (min)
		Ramp Time (min)
		Tray Positions
		Increment Every Runs
		At Time (min)
Separate Power	Separate the sample using power and	Power (W)
Pressure	pressure.	Duration (min)
		Ramp Time (min)
		• Pressure (psi or mbar)
		Pressure Direction
		Tray Positions
		Increment Every Runs
		At Time (min)
	1	1

Table A-1 Time Program Events (continued)

Separate Power       Separate the sample using power and vacuum.       • Power (W)         Vacuum       • Duration (min)         • Ramp Time (min)
Vacuum       • Duration (min)         • Ramp Time (min)
Ramp Time (min)
Vacuum (psi or mbar)
Pressure Direction
Tray Positions
Increment Every Runs
At Time (min)
Separate PressureSeparate the sample using pressure.• Pressure (psi or mbar
Duration (min)
Pressure Direction
Tray Positions
Increment Every Runs
At Time (min)
Separate VacuumSeparate the sample using vacuum.• Vacuum (psi or mbar)
Duration (min)
Pressure Direction
Tray Positions
Increment Every Runs
At Time (min)
Separate Voltage         Separate the sample using voltage.         • Voltage (kV)
Polarity
Duration (min)
Ramp Time (min)
Tray Positions
Increment Every Runs

Table A-1 Time Program Events (continued)

Event	Description	Parameters
Separate Voltage Pressure	Separate the sample using voltage and pressure.	<ul> <li>Voltage (kV)</li> <li>Polarity</li> <li>Duration (min)</li> <li>Ramp Time (min)</li> <li>Pressure (psi or mbar)</li> <li>Pressure Direction</li> <li>Tray Positions</li> <li>Increment Every Runs</li> </ul>
Separate Voltage Vacuum	Separate the sample using voltage and vacuum.	<ul> <li>At Time (min)</li> <li>Voltage (kV)</li> <li>Polarity</li> <li>Duration (min)</li> <li>Ramp Time (min)</li> <li>Vacuum (psi or mbar)</li> <li>Vressure Direction</li> <li>Tray Positions</li> <li>Increment Every Runs</li> <li>At Time (min)</li> </ul>
Stop Data	Stop data collection.	At Time (min)
Wait	Add a wait event.	<ul> <li>Duration (min)</li> <li>Tray Positions</li> <li>Increment Every Runs</li> <li>At Time (min)</li> </ul>

Table A-1 Time Program Events (continued)

Event	Description	Parameters
Wavelength PDA Detector	(PDA detector) Change the wavelength for the specified channel in the PDA detector.	<ul><li>Channel</li><li>Wavelength (nm)</li></ul>
	<b>Note:</b> The wavelength range (wavelength ± ½ bandwidth) must be between 186 nm and 604 nm.	<ul><li>Bandwidth (nm)</li><li>At Time (min)</li></ul>
Wavelength UV Detector	(UV detector) Change the wavelength for Channel 1 in the UV detector.	<ul><li>Wavelength (nm)</li><li>At Time (min)</li></ul>

Table A-1 Time Program Events (continued)

# **Parameters for Time Program Events**

The parameters are listed in alphabetical order.

Table A-2 Parameters for Time Program Events

Parameter	Details	
At Time (min)	The time to start this event, expressed as the time from the first event with the At Time parameter equal to 0.	
Bandwith (nm)	(PDA detector) The bandwidth for a <b>Wavelength PDA Detector</b> event, from 6 nm to 252 nm.	
	Note: The wavelength range (wavelength ± ½ bandwidth) must be between 186 nm and 604 nm.	
Channel	(PDA detector) The channel in the PDA detector to be set to the specified wavelength.	
Current (µA)	The current to be applied during the event, from either $-300.0 \ \mu\text{A}$ to 3.0 $\mu\text{A}$ or 3.0 $\mu\text{A}$ to 300.0 $\mu\text{A}$ .	
	<ul> <li>Values from 3.0 µA to 300.0 µA are normal polarity (+ at the inlet and – at the outlet).</li> </ul>	
	<ul> <li>Values from –300.0 µA to –3.0 µA are reverse polarity (– at the inlet and + at the outlet).</li> </ul>	
Duration (s or min)	The duration of the event.	
	<b>Note:</b> For pressure and vacuum events, the duration must be long enough to allow the system to come to the specified pressure (or vacuum). Refer to About the Duration for Pressure and Vacuum Events.	
Increment Every Runs	The number of runs after which the inlet and outlet vials should increment. Type <b>0</b> if the vial should not increment. Refer to About Vial Incrementing.	
Polarity	The direction of the current to be applied during the event. Options are:	
	• Normal (+): + at the inlet and – at the outlet.	
	• Reverse (-): – at the inlet and + at the outlet.	

Parameter	Details	
Power (W)	The power to be applied during the event, from –9.000 W to 9.000 W.	
	• Values from 0.001 W to 9.000 W are normal polarity (- at the inlet and + at the outlet).	
	• Values from –9.000 W to –0.001 W are reverse polarity (– at the inlet and + at the outlet).	
Pressure (psi or mbar)	The pressure to be applied during the event.	
	<b>Note:</b> The system requires time to come to pressure. If the <b>Duration</b> parameter is too short, the specified pressure can't be reached. Refer to About the Duration for Pressure and Vacuum Events.	
Pressure Direction	The direction of the pressure to be applied during the event. Options are:	
	• Forward: From inlet to outlet.	
	Reverse: From outlet to inlet.	
	Simultaneous: In both directions at once.	
Ramp Time (min)	The time required for the system to reach the specified pressure, voltage, power, or current.	
Relay 1	The relay to be opened or closed.	
Relay 2	The relay to be opened or closed.	
Temperature (°C)	The temperature for the cartridge or sample cooler.	
Tray Positions	The inlet and outlet vials for the event. For each vial, specify a tray and a position. Refer to About Tray Positions.	
Vacuum (psi or mbar)	The vacuum to be applied during the event, from 0.1 psi to 5.0 psi (or 6.9 mbar to 344.7 mbar).	
	<b>Note:</b> The system requires time to come to vacuum. If the <b>Duration</b> parameter is too short, the specified vacuum can't be reached. Refer to About the Duration for Pressure and Vacuum Events.	

Table A-2 Parameters for Time Program Events (continued)

Parameter	Details
Voltage (kV)	The voltage to be applied during the event, from –30.0 kV to 30 kV for any of the <b>Separation Voltage</b> events and –10.0 kV to 10 kV for the <b>Inject Voltage</b> event.
	The direction of the voltage is set by the <b>Polarity</b> parameter.
Wavelength (nm)	The wavelength for the event, from 190 nm to 600 nm.

Table A-2 Parameters for Time Program Events (continued)

### About the Duration for Pressure and Vacuum Events

The system requires time to come to pressure (or vacuum). If the duration is too short, then the specified pressure or vacuum cannot be reached. Use the following tables to make sure that the duration is long enough. Refer to Table A-3 and Table A-4.

To reach this pressure		Set the duration to at least
0.1 psi	6.9 mbar	1.0 sec
0.2 psi	13.8 mbar	1.5 sec
0.3 psi	20.7 mbar	2.0 sec
0.4 psi	27.6 mbar	2.5 sec
0.5 psi	34.5 mbar	3.0 sec
0.7 psi	48.3 mbar	3.4 sec
2.0 psi	137.9 mbar	3.5 sec
5.0 psi	344.7 mbar	3.8 sec
9.5 psi	655.0 mbar	5.0 sec
25.0 psi	1723.7 mbar	6.3 sec

#### Table A-3 Required Duration to Reach Pressure

#### Table A-4 Required Duration to Reach Vacuum

To reach this vacuum		Set the duration to at least
0.10 psi	6.9 mbar	2.0 sec
0.15 psi	10.3 mbar	2.5 sec

To reach this vacuum		Set the duration to at least
0.30 psi	20.7 mbar	3.0 sec
0.40 psi	27.6 mbar	3.5 sec
0.50 psi	34.5 mbar	4.0 sec

Table A-4 Required Duration to Reach Vacuum (continued)

#### **About Tray Positions**

The **Tray Positions** parameter is used to specify the positions of the capillary inlet and outlet for **Rinse**, **Inject**, **Separate**, or **Wait** events.

Parameters for Tray Positions are:

- Inlet Vial: The inlet vial for the next event, from A1 to F6.
- Inlet Tray: The inlet tray for the next event, either Buffer or Sample. For Inject events, Sample List is also available. Refer to Sample Vial Positions for Inject Events.
- **Outlet Vial**: The outlet vial for the next event, from A1 to F6.
- Outlet Tray: The outlet tray for the next event, either Buffer or Sample. For Inject events, Sample List is also available. Refer to Sample Vial Positions for Inject Events.

In the PA 800 Plus System, the geometry of the sample and buffer trays and the dimensions of the capillary cartridge limit access to all 36 positions in the tray. For example, if the capillary inlet is in A6 in the buffer inlet tray, then the capillary outlet cannot access F6 in the buffer outlet tray. These incompatible positions are sometimes referred to as "tray collisions" or "vial collisions".

The software checks the positions and warns the user of any collisions.

Combinations that do not cause a collision are shown in the following table. Refer to Table A-5.

Inlet Columns	Compatible Outlet Columns
A to F	A to C
B to F	A to D
C to F	A to E
D to F	A to F

Table A-5 Inlet and Outlet Columns that Do Not Cause a Collision

#### Sample Vial Positions for Inject Events

The **Inject** event is used to inject the sample to the capillary before the separation begins. The positions of the vials containing the sample for **Inject** events can be specified in the instrument method or in the sample set method.

- 1. To set the vial positions in the instrument method, edit the **Tray Positions** parameter for any **Inject** event.
- 2. To set the vial positions in the sample set method, do the following:
  - a. In the instrument method, select **Sample List** for the **Inlet Tray** in the **Tray Positions** parameter.
  - b. In the sample set method, edit the vial positions in the Plate/Well field.

### **About Vial Incrementing**

Vial incrementing is an automated process to advance the inlet or outlet vials after a specified number of cycles of a method. Vial incrementing eliminates the need to create new methods if different vial positions are needed during the course of a sample set method. Without vial incrementing, vials can overflow with liquid that backs up in the interface block, pressure manifold, and other parts of the system. Additionally, without vial incrementing the ionic strength of the buffer can be depleted.

Vial incrementing is enabled for the **Rinse**, **Inject**, **Separate**, and **Wait** events in an instrument method.

To use vial incrementing, type a value for number of runs in the **Inlet** and **Outlet** fields of the **Increment Every Runs** parameter. Runs are the number of times a method set repeats before vial incrementing occurs.

Vial incrementing restarts when the sample set method advances to a new method set.

This section include the plate definitions for the buffer tray, the sample tray, and the SCIEX 96-well sample plate. These plates must be defined in the Empower<sup>™</sup> Software.

The files should be installed as part of the PA 800 Plus Empower<sup>™</sup> Driver installation.

If they are missing and the plates need to be defined, copy the text, paste it in a text editor, and then save the file.

### **PA800Plus Sample Tray Plate Definition File**

Empower Profile for Plate Type: CE Sample Tray Plate Type: XY Permanent: No Plate Terminology: Plate Well Terminology: Well Plate Dimensions: X: 85.00 Y: 128.00 Height: 17.00 Well Dimensions: Top Left Well X Location: 9.00 Top Left Well Y Location: 17.10 Well Diameter: 12.00 Well Depth: 14.00 Row and Column Dimensions: Number of Rows: 8 Row Spacing: 13.40 mm Number of Columns: 6 Column Spacing: 13.40 mm Row and Column Offsets: Row Offset Type: None Row Offset: 0.00 mm ColumnOffset Type: None Column Offset: 0.00 mm Origin: Bottom Left Scheme: Referencing: XY Horizontal: ABC ... Vertical: 123 ... Sequential Continuous: Off Horizontal First Priority: On

# PA800Plus 96 Well Sample Tray Plate Definition File

```
Empower Profile for Plate Type: 96-Well Sample Tray
Plate Type: XY
Permanent: No
Plate Terminology: Plate
Well Terminology: Well
Plate Dimensions:
 X: 85.00
 Y: 128.00
 Height: 17.00
Well Dimensions:
 Top Left Well X Location: 11.00
 Top Left Well Y Location: 14.50
 Well Diameter: 6.80
 Well Depth: 14.00
Row and Column Dimensions:
 Number of Rows: 12
 Row Spacing: 9.00 mm
 Number of Columns: 8
 Column Spacing: 9.00 mm
Row and Column Offsets:
 Row Offset Type: None
 Row Offset: 0.00 mm
 ColumnOffset Type: None
 Column Offset: 0.00 mm
 Origin: Bottom Left
 Scheme:
 Referencing: XY
 Horizontal: ABC ...
 Vertical: 123 ...
 Sequential Continuous: Off
 Horizontal First Priority: On
```

### **PA800Plus Buffer Tray Plate Definition File**

Empower Profile for Plate Type: CE Buffer Tray Plate Type: XY Permanent: No Plate Terminology: Plate Well Terminology: Well Plate Dimensions: X: 85.00 Y: 85.00 Height: 17.00 Well Dimensions: Top Left Well X Location: 9.00 Top Left Well Y Location: 9.00 Well Diameter: 12.00 Well Depth: 14.00 Row and Column Dimensions: Number of Rows: 6 Row Spacing: 13.40 mm Number of Columns: 6 Column Spacing: 13.40 mm Row and Column Offsets: Row Offset Type: None Row Offset: 0.00 mm ColumnOffset Type: None Column Offset: 0.00 mm Origin: Bottom Left Scheme: Referencing: XY Horizontal: ABC ... Vertical: 123 ... Sequential Continuous: Off Horizontal First Priority: On

# **Topics for Familiarization**

During installation, the FSE should have familiarized or reviewed the following with the customer:

- Software functions:
  - USB license
  - Creating, editing, and saving instrument methods
  - Configuring the software to use multiple plates
  - Direct control of the system, including:
    - Instrument status
    - Status field
    - Parameter tabs and buttons
  - Running a single sample or a sample set method
  - Stopping a run
- Viewing error messages in the Empower<sup>™</sup> Software Message Center window
- Installing a cartridge
- Loading samples
- For systems with more than one detector, changing detectors
- Maintenance procedures

# **Contact Us**

# **Customer Training**

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

# **Online Learning Center**

SCIEX University<sup>™</sup>

### **Purchase Consumables**

Reorder SCIEX consumables online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. The SCIEX online store is currently limited to the US, UK, and Germany but will be expanding to other countries in the future. For customers in other countries, contact the local SCIEX representative.

# **SCIEX** Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
- sciex.com/request-support

# CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

### **Documentation**

This version of the document supercedes all previous versions of this document.

To view this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to https://get.adobe.com/reader.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

To find hardware product documentation, refer to the *Customer Reference* DVD that comes with the system or component.

The latest versions of the documentation are available on the SCIEX website, at sciex.com/customer-documents.

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