SCIEX
The Power of Precision

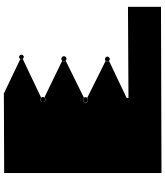
IgG Purity and Heterogeneity Assay Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

RUO-IDV-05-6935-C

July 2020



AB Sciex Pte. Ltd.
Blk33, #04-06 Marsiling Industrial Estate Road 3
Woodlands Central Industrial Estate, Singapore 739256

Contents

IgG Purity and Heterogeneity Assay Kit

The SCIEX IgG Purity and Heterogeneity Assay Kit includes the following:

- reagents and supplies for sample preparation
- methods for a PA 800 Plus Pharmaceutical Analysis System that resolve both reduced and non-reduced immunoglobulins by size, and to quantify heterogeneity and the impurities that might exist in an IgG preparation

This document provides instructions for sample preparation using the IgG Purity and Heterogeneity Assay Kit. It also provides instructions for data acquisition and data analysis using the PA 800 Plus Software and Waters Empower 3 Software (FR4).

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

Note: For accurate results, we strongly recommend using IgG analysis with a PA 800 Plus System that has been qualified with an *Operational Qualification*.

Safety

Refer to the Safety Data Sheets (SDS), available at sciex.com/tech-regulatory, for information about the proper handling of materials and reagents. Always follow standard laboratory safety guidelines. Refer to Hazardous Substance Information for hazardous substances information.

Intended Use

The IgG Purity and Heterogeneity Assay Kit is for laboratory use only.

Introduction

The methodology involves heat denaturing of a specified concentration of protein in the presence of SDS. After being denatured, the sample components are separated by size in a capillary containing a replaceable SDS polymer matrix. The matrix provides the sieving selectivity for the separation.

Two types of analysis methods have been optimized:

- The high-resolution methods use the capillary cartridge in the left to right configuration, with a sample introduction inlet to detection window distance of 20.0 cm.
- The high-speed methods use the capillary cartridge in the right to left configuration, with an inlet to detection window distance of 10 cm.

High-resolution (HR) methods provide high resolution for protein separation (in about 30 minutes). The procedure that follows uses the high-resolution methods.

Note: This application guide has been validated with the PA 800 Plus Pharmaceutical Analysis System.

Internal Standard

A 10 kDa protein Internal Standard is used as a mobility marker. The mobility of all protein samples are calculated relative to this mobility marker allowing for more accurate size estimation and analyte identification.

IgG Control Standard

The IgG Control Standard is an IgG standard that can be used as an experimental control when using the SDS-MW Gel Buffer to determine the purity and heterogeneity of reduced and non-reduced antibody samples.

Sample Buffers

- **SDS-MW Sample Buffer**: The SDS-MW Sample Buffer is provided as part of the IgG Purity and Heterogeneity Assay Kit. This buffer consists of 100 mM Tris-HCl at pH 9.0 with 1% SDS.
- **Low pH SDS sample buffers**: In some cases, a sample buffer with a lower pH (than that of the SDS-MW Sample Buffer) might improve sample stability by minimizing protein degradation. For these samples, the SCIEX low pH SDS sample buffers are available separately:
  - Low pH SDS Sample Buffer: This buffer consists of 100 mM Tris-HCl at pH 6.8 with 1% SDS.
  - Low pH Phosphate SDS Sample Buffer: This buffer consists of 40 mM Phosphate at pH 6.5 with 1% SDS. This buffer meets the *Chinese Pharmacopeia* specification.

Equipment and Materials Required

Note: For items with a reorder part number, sometimes the reorder quantity is different than the quantity in the kit.

Table 1 Kit Contents (PN A10663)

Component	Quantity	Reorder Part Number
Capillary, 50 µm i.d. bare-fused silica	2	338451
SDS-MW Gel Buffer, proprietary formulation, pH 8.0, 0.2% SDS	140 mL, 4-pack	A30341
SDS-MW Sample Buffer, 100 mM Tris-HCl, pH 9.0, 1% SDS	50 mL	N/A
IgG Control Standard, 1 mg/mL	1 mL, 3-pack	391734
Internal Standard, 10 kDa protein, 5 mg/mL	0.4 mL	A26487
Acid Wash/Regenerating Solution, 0.1 M HCl	100 mL	N/A
Basic Wash Solution, 0.1 M NaOH	100 mL	338424

Table 2 Additional Supplies from SCIEX

Component	Quantity	Part Number
(Optional) Low pH SDS Sample Buffer, 100 mM Tris-HCl, pH 6.8, 1% SDS	140 mL	C44807
(Optional) Low pH Phosphate SDS Sample Buffer, 40 mM Phosphate, pH 6.5, 1% SDS	140 mL	C57805
Micro Vials, 200 µL	100	144709
Universal Vial Caps, blue	100	A62250
Universal Vials	100	A62251

Table 3 Additional Required Reagents or Supplies

Description	Vendor	Part Number
2-mercaptoethanol	Sigma-Aldrich	M7154
Iodoacetamide	Sigma-Aldrich	I-1149
(Optional) Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-30 membrane	Millipore	UFC803096

Storage Conditions

- Upon receipt, store the 10 kDa Internal Standard at 2 °C to 8 °C.
- Upon receipt, prepare 95 µL aliquots of the IgG Control Standard and store them promptly at –20 °C.
- Store the capillary, SDS-MW Sample Buffer, SDS-MW Gel Buffer, Acid Wash/Regenerating Solution, and Basic Wash Solution at room temperature.
- Store the Low pH SDS Sample Buffer and Low pH Phosphate SDS Sample Buffer at room temperature.

Note: If the gel buffer or sample buffers are refrigerated, then precipitates might form. If precipitates are present, stir the buffer until the precipitate is fully dissolved before using.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat
- Table-top mini centrifuge
- Microcentrifuge, or equivalent, and microcentrifuge tubes
- Water bath or heat block, 37 °C to 100 °C
- Vortex mixer
- Centrifugal vacuum evaporator
- Analytical balance
- Pipettes and appropriate tips
- Parafilm
- Spatula
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2 µm filter and with resistance greater than 18 MΩ)

Required Detector

A photodiode array (PDA) detector is required.

Required Cartridge or Capillary

One of the following:

- Pre-assembled cartridge (PN A55625)
- Capillary cartridge (PN 144738) and capillaries, bare-fused silica, 50 µm i.d. (PN 338451)

Methods and Sequences

Note: The following information applies to users using the PA 800 Plus System with the PA 800 Plus and 32 Karat™ Software. If the system will be used with the Empower™ Software, then the methods are different. Refer to Run the Samples with the Waters Empower™ Software.

The methods and sequences files are installed on the PA 800 Plus controller. They are not available for download. The methods and sequences can also be created manually. Refer to Methods.

The following methods are in the PA 800 Plus controller at C:\32Karat\projects\IgG Purity\Method.

- **IgG HR Conditioning - PA 800 plus.met**: Conditions the capillary at the start of each day. In the method names, HR indicates high resolution and HS indicates high speed.
- **IgG HR Separation - PA 800 plus.met**: Performs an IgG separation.
- **IgG HR Shutdown - PA 800 plus.met**: Shuts down and cleans the capillary at the end of a sequence, rinses the capillary for storage, and then turns off the UV lamp or, in the PDA detector, turns off the laser.
- **IgG HS Conditioning - PA 800 plus.met**: Conditions the capillary at the start of each day.
- **IgG HS Separation - PA 800 plus.met**: Performs an IgG separation.
- **IgG HS Shutdown - PA 800 plus.met**: Shuts down and cleans the capillary at the end of a sequence, rinses the capillary for storage, and then turns off the UV lamp or, in the PDA detector, turns off the laser.

The following sequences are in the PA 800 Plus controller at C:\32Karat\projects\IgG Purity\Sequence.

- **IgG HR - 24 samples - PA 800 plus.seq:** Contains the sequence table with high-resolution (HR) methods and can run up to 24 samples where sample number 1 is (always) the IgG Control Standard.
- **IgG HR - PA 800 plus.seq**: Contains the sequence table with high-resolution (HR) methods and can run up to 8 samples of the IgG Control Standard for troubleshooting purposes or can be changed to your desired number of samples with proper sample ID and used where sample number 1 is (always) the IgG Control Standard.
- **IgG HS - PA 800 plus.seq**: Contains the sequence table with high-speed (HS) methods, and can run up to 8 samples of the IgG Control Standard or can be modified to include customer samples.

Prepare the Samples

Prepare the IgG Control Standard (Reduced)

1 Thaw one of the 95 µL aliquots of the IgG Control Standard at room temperature.

2 Add 2 µL of the 10 kDa Internal Standard to the IgG tube.

3 Inside a fume hood, add 5 µL of 2-mercaptoethanol to the IgG tube.

4 Cap the tube and then mix thoroughly.

5 Using a centrifuge, spin the tube for 1 minute at 300 *g*.

6 Seal the vial cap with Parafilm and then heat the vial at 70 °C for 10 minutes.

7 Allow the tube to cool down to room temperature for at least 3 minutes.

8 Using a centrifuge, spin the tube for 1 minute at 300 *g* to collect all liquid to the bottom of the tube.

9 Transfer 70 µL to 90 µL of the prepared sample to a micro vial, put the micro vial inside a universal vial, then cap the universal vial.

Prepare the IgG Control Standard (Non-reduced)

Before preparing the IgG non-reduced control standard, prepare a 250 mM iodoacetamide (IAM) solution. The IAM solution acts as the alkylation reagent during preparation of the IgG non-reduced control standard. The IAM solution is stable for approximately 24 hours at room temperature.

Prepare the Alkylation Reagent (250 mM IAM Solution)

1 Weigh 46 mg of iodoacetamide (IAM).

2 Transfer the IAM to a 1.5 mL centrifuge tube.

3 Add 1 mL of DDI water to the 1.5 mL centrifuge tube.

4 Cap the vial tightly, mix until dissolved, and then store the vial in the dark at room temperature. The solution is stable for approximately 24 hours at room temperature.

Prepare the Non-reduced IgG Control Standard

1 Thaw one of the 95 µL aliquots of the IgG Control Standard at room temperature.

2 Add 2 µL of the 10 kDa Internal Standard to the IgG Control Standard tube.

3 Add 5 µL of a 250 mM IAM solution.

4 Cap the tube and then mix throughly.

5 Using a centrifuge, spin the tube for 1 minute at 300 *g*.

6 Seal the tube with Parafilm and then heat the tube at 70 °C for 10 minutes.

7 Allow the tube to cool down to room temperature for at least 3 minutes.

8 Using a centrifuge, spin the tube for 1 minute at 300 *g* to collect all liquid to the bottom of the tube.

9 Transfer 70 µL to 90 µL of the prepared sample to a micro vial. Put the micro vial inside a universal vial, then cap the universal vial.

Prepare the IgG Sample

Note: If the sample concentration is less than 10 mg/mL and the buffer concentration is more than 50 mM, then use the Amicon Ultra-4 centrifugal filter unit to exchange the buffer with the SDS-MW Sample Buffer. Refer to Perform a Buffer Exchange for the IgG Sample.

Prepare the Reduced IgG Sample

1 Pipette 100 µg of IgG sample in a volume less than 45 µL to a 0.5 mL microcentrifuge tube.

2 Add 50 µL to 95 µL of SDS-MW Sample Buffer to give a final volume of 95 µL.

3 Add 2 µL of the 10 kDa Internal Standard to the IgG sample tube.

4 Inside a fume hood, add 5 µL of 2-mercaptoethanol to the IgG sample tube.

5 Cap the tube tightly and mix thoroughly.

6 Using a centrifuge, spin the tube for 1 minute at 300 *g*.

7 Seal the tube with Parafilm and then heat the tube in a water bath at 70 °C for 10 minutes.

8 Allow the tube to cool down to room temperature for at least 3 minutes.

9 Using a centrifuge, spin the tube for 1 minute at 300 *g* to collect all liquid to the bottom of the tube.

10 Transfer 70 µL to 90 µL of the prepared sample to a micro vial. Put the micro vial inside a universal vial, then cap the universal vial. Make sure there are no bubbles in the micro vial.

Tip! To remove bubbles from the micro vial, use a pipette to gently aspirate any bubbles.

Prepare the Non-reduced IgG Sample

Under non-reduced conditions, heating the sample solution at high temperature is required to accelerate SDS binding. However, heating an IgG sample at high temperature might introduce fragmentation and aggregation, and introduce artifacts to the sample analysis.

To alleviate these temperature-induced artifacts, first alkylate the IgG sample using the following procedure.

1 Pipette 100 µg of IgG sample to a 0.5 mL microcentrifuge tube.

2 Add 50 µL to 95 µL of SDS-MW Sample Buffer to give a final volume of 95 µL.

3 Add 2 µL of the 10 kDa Internal Standard to the IgG sample tube.

4 Inside a fume hood, add 5 µL of the 250 mM IAM solution to the IgG sample tube. Refer to Prepare the Alkylation Reagent (250 mM IAM Solution).

5 Cap the tube tightly and mix thoroughly.

6 Using a centrifuge, spin the tube for 1 minute at 300 *g*.

7 Seal the tube with Parafilm and then heat the mixture in a water bath at 70 °C for 10 minutes.

8 Allow the tube to cool down to room temperature for at least 3 minutes.

9 Using a centrifuge, spin the tube for 1 minute at 300 *g* to collect all liquid to the bottom of the tube.

10 Transfer 70 µL to 90 µL of the prepared sample to a micro vial. Put the micro vial inside a universal vial, then cap the universal vial. Make sure there are no bubbles in the micro vial.

Tip! To remove bubbles from the micro vial, use a pipette to gently aspirate any bubbles.

Perform a Buffer Exchange for the IgG Sample

Note: The signal intensity and resolution of this assay is sensitive to the salt concentration in the IgG sample. If the salt concentration is too high, then low signal and peak tailing is likely to occur. Exchange the SDS-MW Sample Buffer with an Amicon Ultra-4 centrifugal filter unit using the following procedure.

Note: For desalting/buffer exchange procedures using a device from another vendor, read the user guide from the vendor before use.

1 Add 1 mL of IgG sample to filter unit.

2 Using a centrifuge, spin the sample for 15 minutes at 4,000 *g*. Discard the filtrate.

3 Add 2 mL of SDS-MW Sample Buffer, then use a centrifuge and spin for 25 minutes at 4,000 *g*.

4 Put the filter unit inside a new vial in an inverted position and then use a centrifuge to spin the vial for 3 minutes at 1,000 *g*. The IgG solution will collect in the vial.

5 Transfer the collected protein to an appropriate sterile tube. Add SDS-MW Sample Buffer to give a final volume of 1 mL.

Prepare the PA 800 Plus System

This section describes the steps to prepare the PA 800 Plus System to acquire data.

The procedures described in this section assume the system has already been properly installed and initialized.

Install the PDA Detector

1 Turn off the PA 800 Plus System and then install the PDA detector. Refer to the *System Maintenance Guide*.

2 Turn on the system and permit the UV lamp to warm up for at least 30 minutes.

Clean the Interface Block

CAUTION: Potential System Damage. Do not allow the gel to accumulate on the electrodes, opening levers, capillary ends, and interface block. Gel accumulation might cause broken capillaries, bent electrodes, jammed vials, or missed injections.

Clean the electrodes, opening levers, capillary tips, and interface block weekly or when changing chemistries. Refer to the *System Maintenance Guide* for detailed instructions.

The SDS-MW Gel Buffer is very viscous and will accumulate on the capillary ends, electrodes, interface block, and opening levers unless regular and thorough cleaning is performed.

Install the Cartridge and Calibrate the Detector

Note: To make sure that the 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.

Note: For Empower™ Software users, calibration instructions are located in the *PA 800 Plus Empower™ Driver User Guide*.

1 Remove the cartridge from the box and, if necessary, install the capillary.

2 Install the cartridge in the PA 800 Plus System. Refer to the *System Maintenance Guide*.

3 Close the front panel.

4 Calibrate the detector.

Click the rainbow above the lamp in the Direct Control window to open the PDA Detector Parameters dialog and then click **Calibrate**. Refer to the *System Maintenance Guide*.

Load the Buffer Trays

DANGER! Toxic Chemical Hazard. Read the *Safety Data Sheets* for Acid Wash/Regenerating Solution (0.1 M HCl), Basic Wash Solution (0.1 M NaOH), and SDS-MW Gel Buffer before use.

Refer to Hazardous Substance Information for additional information.

Note: Before starting the separation, bring the SDS-MW Gel Buffer and the SDS-MW Sample Buffer to room temperature.

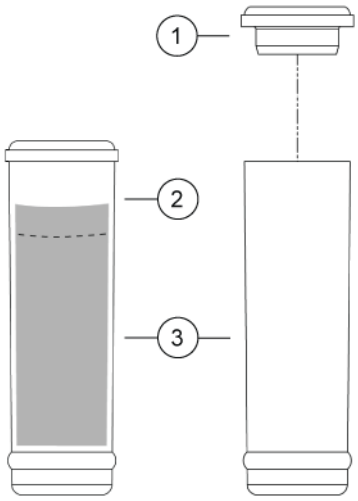
Note: Make sure to fill the buffer vials with SDS-MW Gel Buffer without producing bubbles and use the recommended volume. If the volume is too low (less than half of the vial volume), then the capillary and electrode might not be able to dip in the SDS-MW Gel Buffer during the separation. If the volume is too high, then the SDS-MW Gel Buffer might accumulate on the capillary ends and electrodes, resulting in system failure.

1 Depending on the number of samples to be run, fill the appropriate number of vials and then cap them with the blue caps. For each set of eight samples, prepare the following:

- 3 universal vials, containing 1.2 mL of SDS-MW Gel Buffer, for the Gel-R positions
- 3 universal vials, containing 1.1 mL of SDS-MW Gel Buffer, for the Gel-S positions
- 12 universal vials, containing 1.5 mL of DDI water, for the H_2O positions
- 3 universal vials, containing 1.5 mL of Acid Wash/Regenerating Solution, for the HCl positions
- 3 universal vials, containing 1.5 mL of Basic Wash Solution, for the NaOH positions
- 9 universal vials, containing 1.0 mL of DDI water, for the Waste positions in the outlet buffer tray

CAUTION: Potential System Damage. Do not fill the waste vial with more than 1.8 mL. If the vial has more than 1.8 mL, the pressure system might be damaged.

Figure 1 Universal Vial and Cap Setup



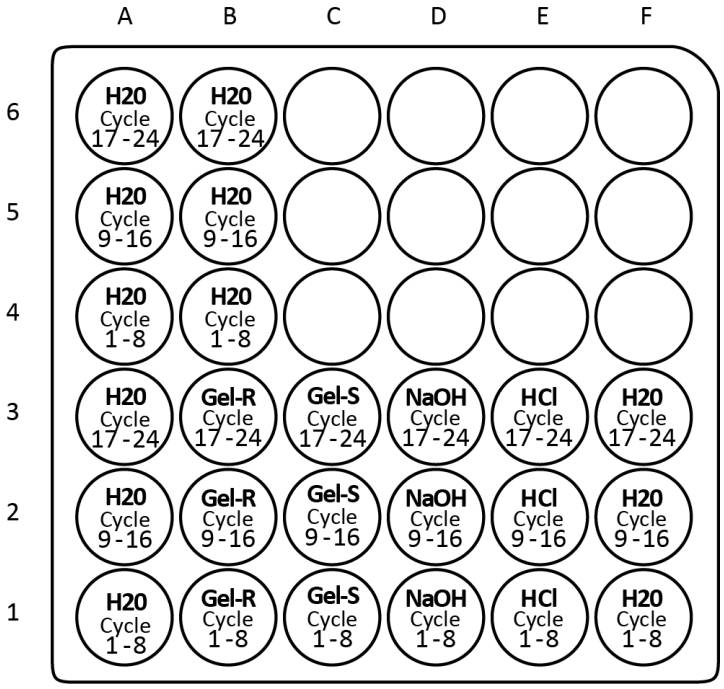
1. Universal vial cap
2. Maximum fill line
3. Universal vial

2 Put the vials in the buffer trays. Refer to Figure 2 and Figure 3.

Note: The vial positions in the following figures are for the high-resolution sequence. If the high-speed sequence is being used, then reverse the positions of the vials. Refer to Figure 4.

IMPORTANT For this application, all vials and caps are designed for a maximum of eight runs. Do not reuse the caps because they might be contaminated with dried gel and other chemicals.

Figure 2 Inlet Buffer Tray Layout



A1 to A6: 1.5 mL DDI H_2O, used in dip step to clean capillary tip

B4 to B6: 1.5 mL DDI H_2O, used in dip step to clean capillary tip

B1 to B3: 1.2 mL SDS-MW Gel Buffer, used to rinse/fill capillary prior each cycle (Gel-R)

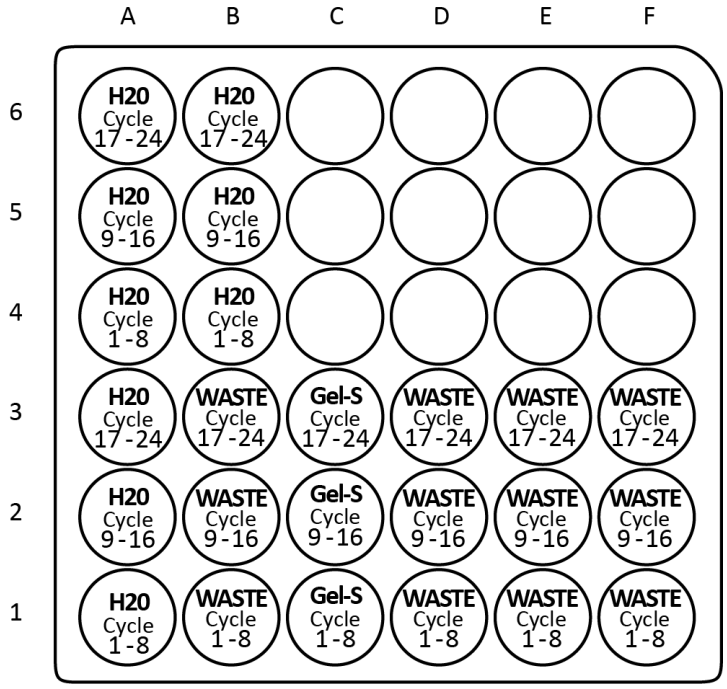
C1 to C3: 1.1 mL SDS-MW Gel Buffer, used for separation (Gel-S)

D1 to D3: 1.5 mL 0.1 M NaOH solution, used to precondition capillary

E1 to E3: 1.5 mL 0.1 M HCl solution, used to precondition capillary

F1 to F3: 1.5 mL DDI H_2O, used to precondition capillary

Figure 3 Outlet Buffer Tray Layout



A1 to A6: 1.5 mL DDI H_2O, used in dip step to clean capillary tip

B4 to B6: 1.5 mL DDI H_2O, used in dip step to clean capillary tip

B1 to B3: 1.0 mL DDI H_2O, waste for SDS-MW Gel Buffer rinse

C1 to C3: 1.1 mL SDS-MW Gel Buffer, used for separation

D1 to D3: 1.0 mL DDI H_2O, waste for 0.1 M NaOH solution rinse

E1 to E3: 1.0 mL DDI H_2O, waste for 0.1 M HCl solution rinse

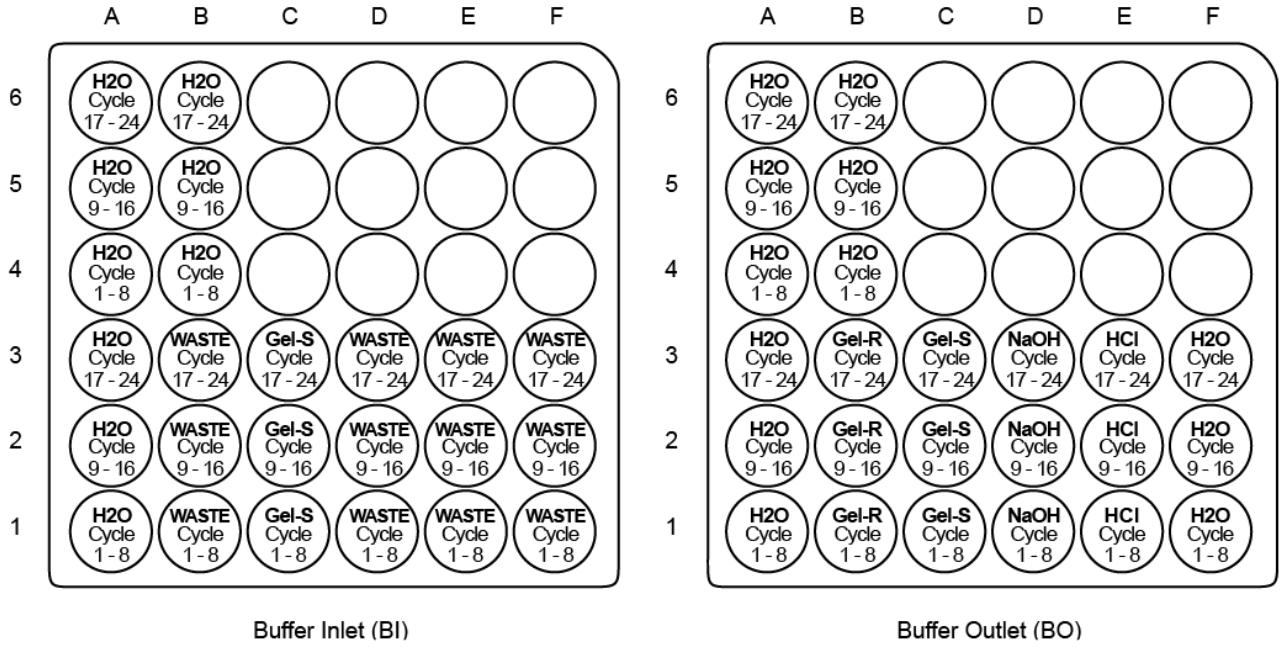
F1 to F3: 1.0 mL DDI H_2O, waste for DDI H_2O rinse

Note: During electrophoresis the ionic strength of the buffer will change. The separation method is programmed to increment the buffer vials after eight runs to avoid ionic depletion.

Buffer Trays for a High-speed Separation

For high-speed separations, put the vials in the inlet and outlet buffer trays as shown in Figure 4.

Figure 4 Buffer Tray Layout for High-speed Separations



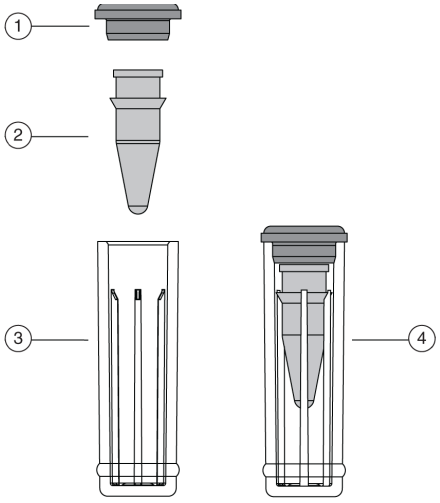
Load the Sample Tray

1 Prepare the samples. For each sample:

- **a.** Make sure the prepared samples are at room temperature.
- **b.** Put 70 µL to 90 µL of the sample in a micro vial.
- **c.** Make sure that no bubbles are present at the bottom of the vials. If bubbles are present, then use a centrifuge to spin the micro vials for 2 minutes at 1,000 *g*. Repeat if necessary.

2 Put the micro vial in a universal vial, and then cap the universal vial.

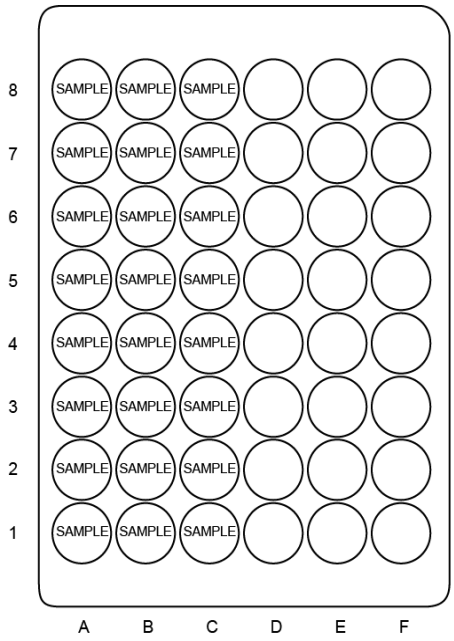
Figure 5 Sample Vial Setup



1. Universal vial cap	**3.** Universal vial
2. Micro vial	**4.** Micro vial inside universal vial

3 Put the universal vials in positions A1:C8 in the sample tray. Refer to Figure 6. For fewer than 24 samples, start at position A1 and fill all the A wells before filling any other wells.

Figure 6 Sample Tray Layout



Run the Samples

Create the Sequence and Start the Run

Note: For Empower™ Software users, refer to Run the Samples with the Waters Empower™ Software.

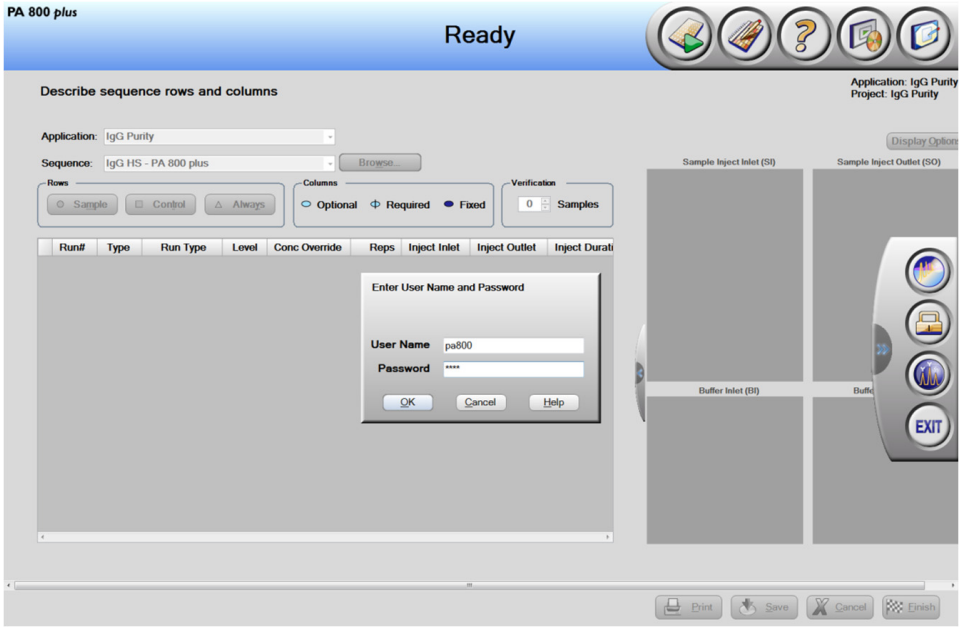
1 Double-click the PA 800 Plus Software icon on the desktop.

2 In the PA 800 Plus window, click  (**Run**) in the upper right corner of the window.

3 In the **Application** list, click **IgG Purity.** In the **Sequence** list, click **Browse** and select **IgG HS - PA 800 plus**.

If system administration is enabled, type a user name and password when prompted, and then click **OK**. Refer to Figure 7. The default user name is **pa800**, and the default password is **plus.**

Figure 7 User Name and Password Entry



The **Instrument Status and Direct Control** window opens. Refer to Figure 8.

Figure 8 Instrument Status and Direct Control Window: Idle

4 In the **Instrument Status and Direct Control** window, click Next (**Next**) in the bottom right corner of the window.

The sequence opens.

5 Select **IgG HS - PA 800 plus** to open the sequence. This sequence will run a maximum of 24 samples where sample 1 is (always) the IgG Control Standard.

6 Click (**Describe**) in the upper right corner of the window to edit the sequence.

Use the Describe function to customize the sequence table and edit the number of samples that can be run in the sequence. The Describe function can also be used to set the row types.

7 In the **Application** list, click **IgG Purity.** In the **Sequence** list, click **Browse**, and then select **IgG HS - PA 800 plus**. If prompted, type a user name and password.

The page updates to show the selected sequence, and all rows in the sequence are designated as samples.

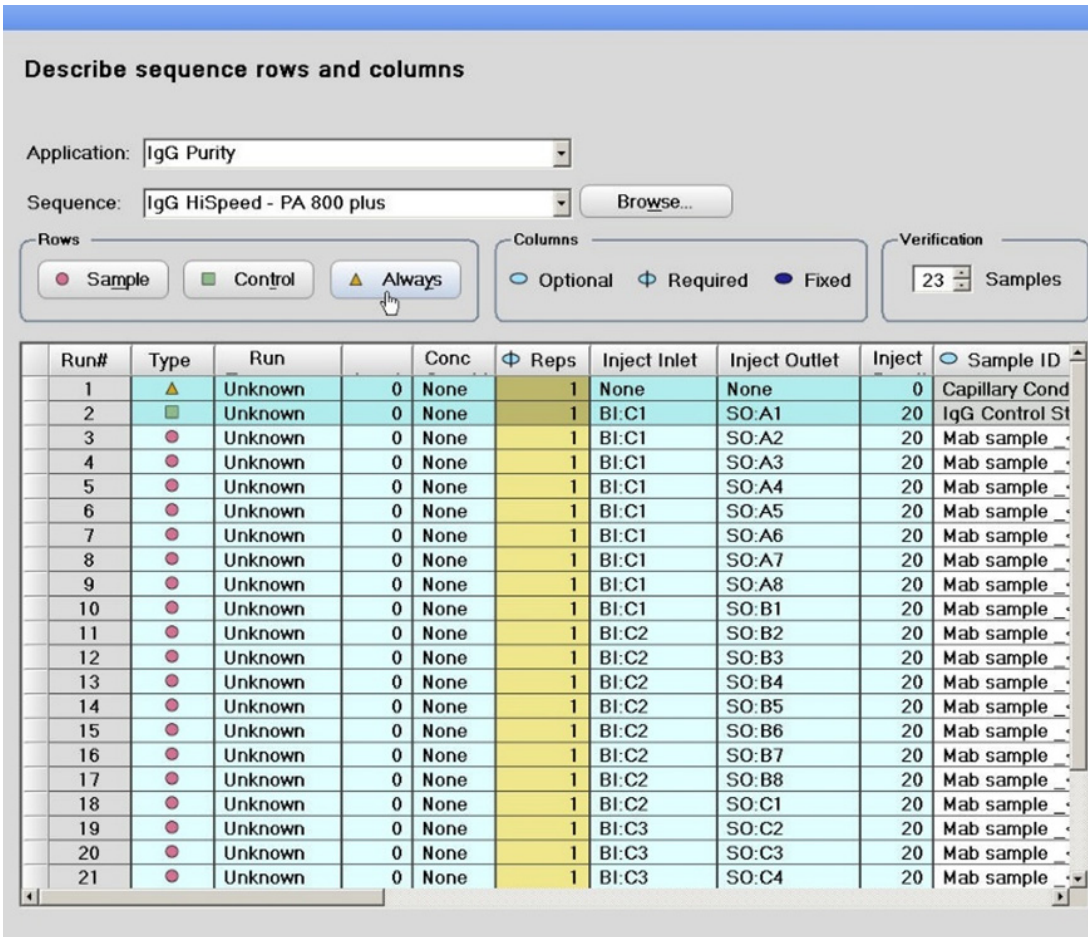
8 (Optional) Edit the **Sample ID** and the **Data File Name** as desired.

Editable fields such as **Sample ID** and **Data File Name** can be set as Mandatory, Optional, or Fixed.

9 After the sequence is loaded, set rows to **Sample**, **Control**, or **Always**. Click a row to select it and then click the button in the **Rows** area.

Capillary Conditioning and **Shutdown** runs are set as **Always.** Refer to Figure 9. Run #2 is set as **Control.** **Sample ID** is set as **Optional. Reps** are set as **Required.**

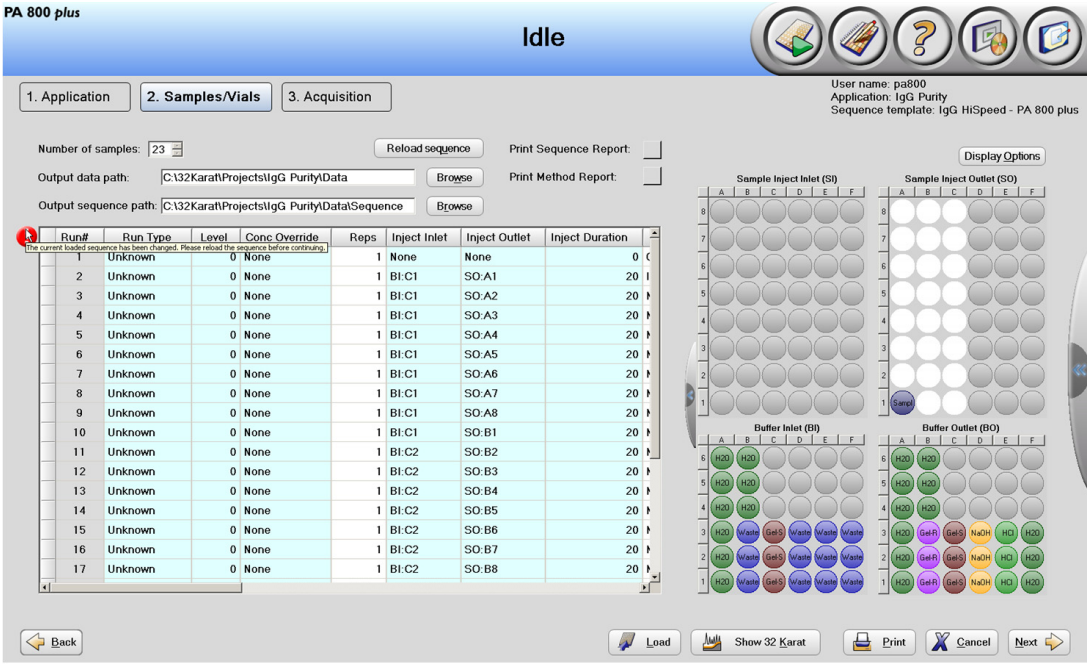
Figure 9 Describe sequence rows and columns Window – Conditioning Method Set to "Always"



Run#	Type	Run		Conc	Reps	Inject Inlet	Inject Outlet	Inject	Sample ID
1	▲	Unknown	0	None	1	None	None	0	Capillary Cond
2	■	Unknown	0	None	1	BI:C1	SO:A1	20	IgG Control St
3	●	Unknown	0	None	1	BI:C1	SO:A2	20	Mab sample _
4	●	Unknown	0	None	1	BI:C1	SO:A3	20	Mab sample _
5	●	Unknown	0	None	1	BI:C1	SO:A4	20	Mab sample _
6	●	Unknown	0	None	1	BI:C1	SO:A5	20	Mab sample _
7	●	Unknown	0	None	1	BI:C1	SO:A6	20	Mab sample _
8	●	Unknown	0	None	1	BI:C1	SO:A7	20	Mab sample _
9	●	Unknown	0	None	1	BI:C1	SO:A8	20	Mab sample _
10	●	Unknown	0	None	1	BI:C1	SO:B1	20	Mab sample _
11	●	Unknown	0	None	1	BI:C2	SO:B2	20	Mab sample _
12	●	Unknown	0	None	1	BI:C2	SO:B3	20	Mab sample _
13	●	Unknown	0	None	1	BI:C2	SO:B4	20	Mab sample _
14	●	Unknown	0	None	1	BI:C2	SO:B5	20	Mab sample _
15	●	Unknown	0	None	1	BI:C2	SO:B6	20	Mab sample _
16	●	Unknown	0	None	1	BI:C2	SO:B7	20	Mab sample _
17	●	Unknown	0	None	1	BI:C2	SO:B8	20	Mab sample _
18	●	Unknown	0	None	1	BI:C2	SO:C1	20	Mab sample _
19	●	Unknown	0	None	1	BI:C3	SO:C2	20	Mab sample _
20	●	Unknown	0	None	1	BI:C3	SO:C3	20	Mab sample _
21	●	Unknown	0	None	1	BI:C3	SO:C4	20	Mab sample _

10 In the lower right corner of the window, click Save (**Save**) and then click Finish (**Finish**). The Run Sequence window opens. Refer to Figure 10.

Figure 10 Describe sequence rows and columns Window – Reload Sequence



Note: In the upper left corner next to Run #1 of the table in Figure 10, a blinking red exclamation mark indicates that the sequence has changed and that the software expects an action from the user. Move the cursor over the exclamation point to view a tooltip with the required action. In this example, the user is prompted to click Reload sequence (**Reload sequence**) to update the sequence.

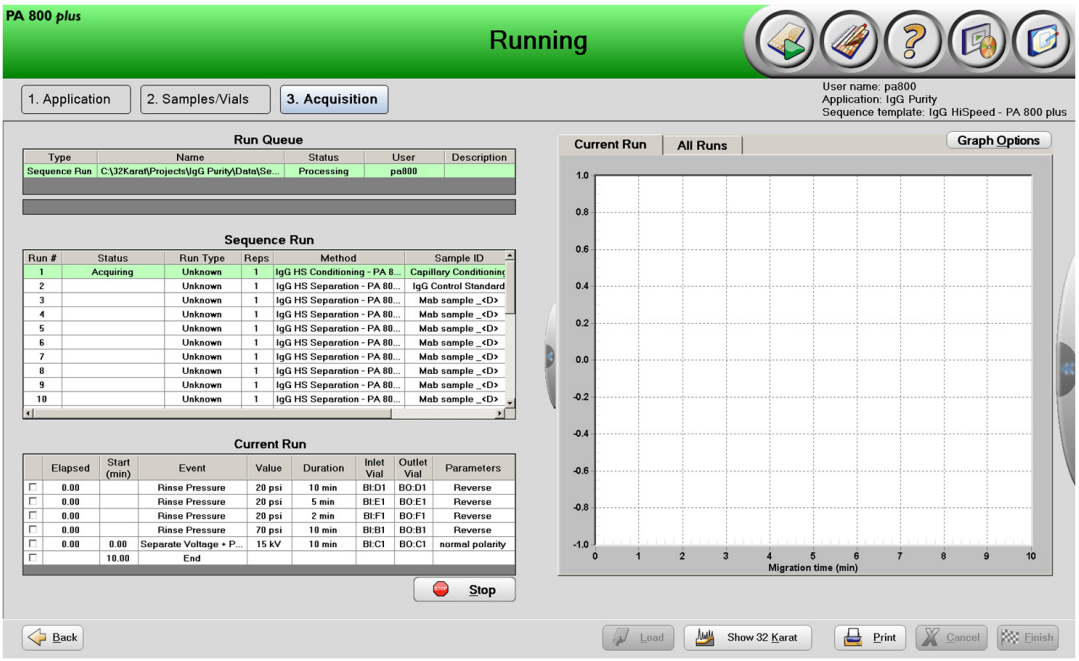
A required action is also used if the data file name is a required field, and the data file does not contain any information. In this case, the user is required to enter the appropriate data file name.

The number of samples shown for this sequence is 23 instead of 24 because the first run is the control. If required, the user can reduce the number the samples to be run in the sequence in the **Run Sequence** window by editing the **Number of samples** list.

11 Click **Load** to load the sample and reagent vials as shown in Figure 10 and then close the door.

12 Click Next (**Next**) and then click **Yes - run now.**

Figure 11 PA 800 Plus System During Data Acquisition



Waste Disposal

WARNING! Biohazard or Toxic Chemical Hazard. Follow local directives when disposing of chemicals, vials and caps, and the remains of the prepared samples, if applicable. They might contain regulated compounds and biohazardous agents.

Store the Cartridge

Store the Cartridge for Less Than 10 Days

1 Perform a shutdown method to clean the capillary.

The shutdown method fills the capillaries with water.

2 Store the cartridge for up to 10 days in the system with the capillary ends immersed in vials of DDI water.

Store the Cartridge for More Than 10 Days

1 Perform a shutdown method to clean the capillary.

2 Rinse the capillary with DDI water for 10 minutes at 100 psi.

3 Remove the cartridge from the system.

4 Store the cartridge upright in the cartridge box at room temperature, with the capillary ends immersed in vials of DDI water.

Prepare the Cartridge After Storage

- If the cartridge has not been used for more than a day or it has been stored for an extended time, then condition the capillary using the IgG Conditioning method.

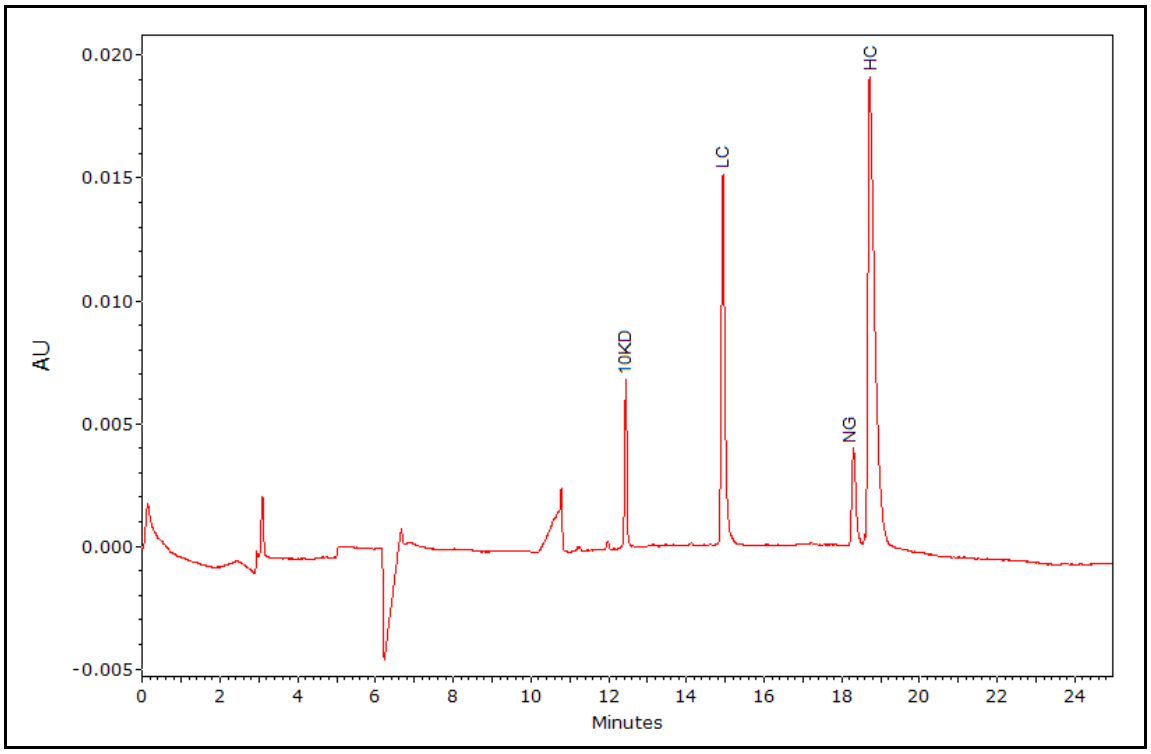
Analyze the Results

Typical Results for the Reduced IgG Control Standard

The IgG Control Standard used with this assay includes a controlled percentage of non-glycosylated heavy chain, which provides both a resolution and a quantification benchmark. Figure 12 illustrates a typical electropherogram of the reduced IgG control standard for the suitability test.

With this assay, the suitability standard is used to confirm the identification of the known IgG control elements of the IgG light chain (LC), the heavy chain (HC), the non-glycosylated heavy chain (NG), and the 10 kDa Internal Standard (10 kD). The glycosylated heavy chain should be baseline resolved from the non-glycosylated heavy chain (resolution > 1). The quantification benchmark is given as the percentage of the total heavy chain present in the IgG control. Refer to the *Certificate of Analysis* shipped with the Internal Standard for the percentage.

Figure 12 Electropherogram of IgG Control Standard Using High-resolution Methods Under Reducing Conditions

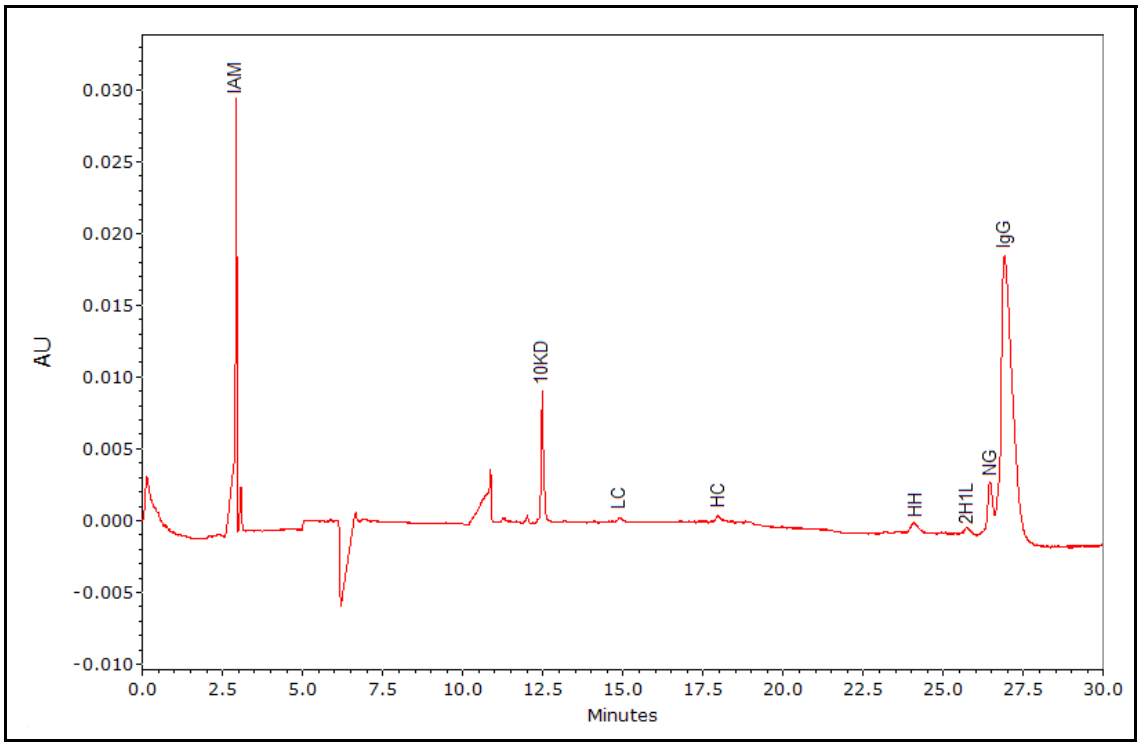


- 10KD: 10 kDA Internal Standard
- LC: light chain
- NG: non-glycosylated heavy chain
- HC: heavy chain

Typical Results for the Non-reduced IgG Control Standard

Under non-reducing conditions, all of the impurities, such as the light chain (LC), the heavy chain (HC), the heavy-heavy chain (HH), and the 2 heavy 1 light chain (2H1L), resolve from the whole antibody.

Figure 13 Electropherogram of IgG Control Standard Using High-resolution Methods Under Non-reducing Conditions



- 10KD: 10 kDA Internal Standard
- LC: light chain
- HC: heavy chain
- HH: heavy-heavy chain
- 2H1L: 2 heavy 1 light chain
- NG: non-glycosylated heavy chain

Troubleshooting

Table 4 Troubleshooting

Symptom	Possible Cause	Corrective Action
Failed system suitability test	Incorrect capillary length or improperly-positioned reagents and samples	Inspect the capillary length, buffer tray, and sample tray first. Make sure that all the reagents and samples are in the right place as described in the buffer tray maps. Evaluate the integration and peak identification windows. Refer to the other symptoms below for additional troubleshooting tips.
Shifts in migration with low or erratic current	Plugged capillary	1) Rinse the capillary with DDI water at 100 psi for 10 minutes and then perform the capillary conditioning method. 2) If low or unsteady current continues, replace the capillary.
	Air bubbles in the gel	Degas SDS-MW Gel Buffer under 5 Hg to 15 Hg vacuum for 5 minutes.
	Contaminated electrode	Clean electrodes. Refer to the *System Maintenance Guide* for further instructions.
High current	Contaminated gel buffer	Replace the SDS-MW Gel Buffer as needed.
	Contamination of the electrode	Clean the electrodes. Refer to the *System Maintenance Guide*.
Spikes in electropherogram	Air bubbles in gel buffer	Degas SDS-MW Gel Buffer under a 5 Hg to 15 Hg vacuum for 5 minutes.
Broad peaks, poor resolution	Poor capillary end cut	Inspect the capillary end under magnification. If the cut is jagged, then cut the end again, or replace the capillary.
	Improper reduction of sample	Reduce the sample using the recommended procedure. Use fresh 2-mercaptoethanol for sample reduction. Refer to Prepare the Reduced IgG Sample.
	Deteriorated capillary	1) Rinse the capillary with DDI water for 10 minutes at 100 psi and then perform the capillary conditioning method. 2) Install a new capillary if the same problem is observed.
	Dust or gel build up on capillary end	Clean the capillary tip using DDI water. Refer to Clean the Interface Block.

Table 4 Troubleshooting (Continued)

Symptom	Possible Cause	Corrective Action
No peaks or low signal	Capillary inlet longer than the inlet electrode	Push the capillary up inside the cartridge or cut the capillary inlet to make sure that it is the same length as the electrode.
	Dirty or plugged capillary tip	1) Clean the capillary tip using DDI water. Refer to Clean the Interface Block. 2) Replace the capillary if the plug cannot be removed by water rinses.
	Not enough sample	Make sure there is a minimum of 20 µL of sample in the sample vial.
	Slow sample migration	Increase the separation time in the method and repeat the analysis.
	High salt in IgG sample	Perform a buffer exchange to remove salt from the sample. Refer to Perform a Buffer Exchange for the IgG Sample.

APPENDIX A

Hazardous Substance Information

The following information must be noted and the relevant safety measures taken. Refer to the respective safety data sheets for more information. These are available upon request or can be downloaded from our website, sciex.com/tech-regulatory.

Hazard classification is according to HCS 2012.

Acid Wash/Regenerating Solution (0.1 M HCl)

DANGER! Causes severe skin burns and eye damage.

Basic Wash Solution (0.1 M NaOH)

DANGER! Causes severe skin burns and eye damage.

Low pH SDS Sample Buffer (100 mM Tris-HCl, pH 6.8, 1% SDS)

WARNING! Causes mild skin irritation.

Low pH Phosphate SDS Sample Buffer (40 mM Phosphate, pH 6.5, 1% SDS)

WARNING! Causes mild skin irritation.

IgG Control Standard

WARNING! Causes mild skin irritation.

SDS-MW Sample Buffer (100 mM Tris-HCl, pH 9.0, 1% SDS)

WARNING! Causes mild skin irritation.

SDS-MW Gel Buffer, proprietary formulation (pH 8.0, 0.2% SDS)

DANGER! Causes mild skin irritation. May damage fertility or the unborn child.

Other Reagents

These components are not classified as hazardous:

- Internal Standard, 10 kDa protein, 5 mg/mL

For reagents from other vendors, read the Safety Data Sheet from the vendor before use.

APPENDIX B

Methods

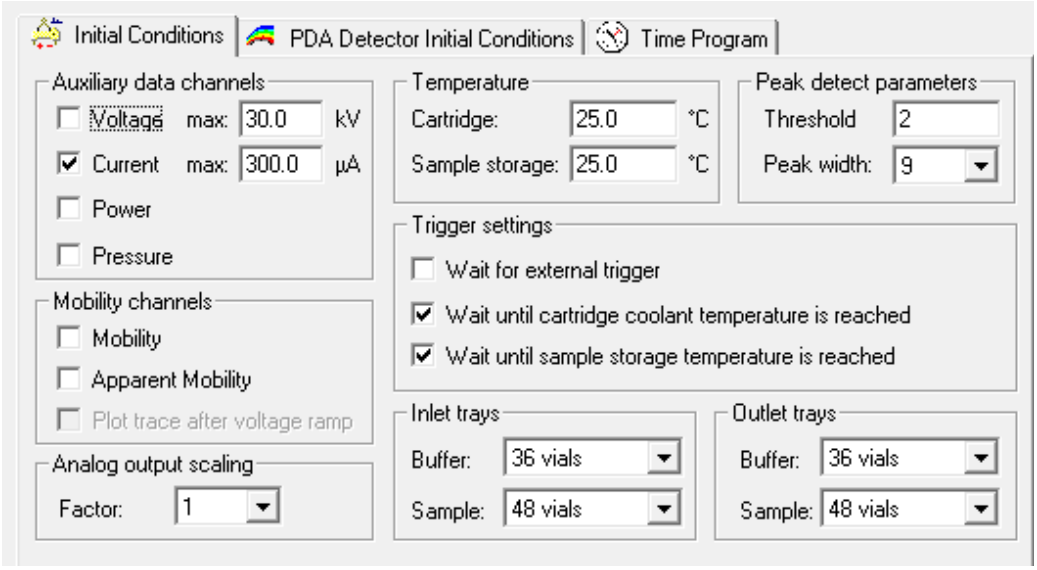
The IgG analysis requires three methods. Two types of separations are possible: high resolution and high speed.

Note: The following information applies to users using the PA 800 Plus System with the PA 800 Plus and 32 Karat™ Software. If the system will be used with the Empower™ Software, then the methods will be different. Refer to Run the Samples with the Waters Empower™ Software.

Note: The values on the Initial Conditions and PDA Detector Initial Conditions tabs are the same for all of the methods.

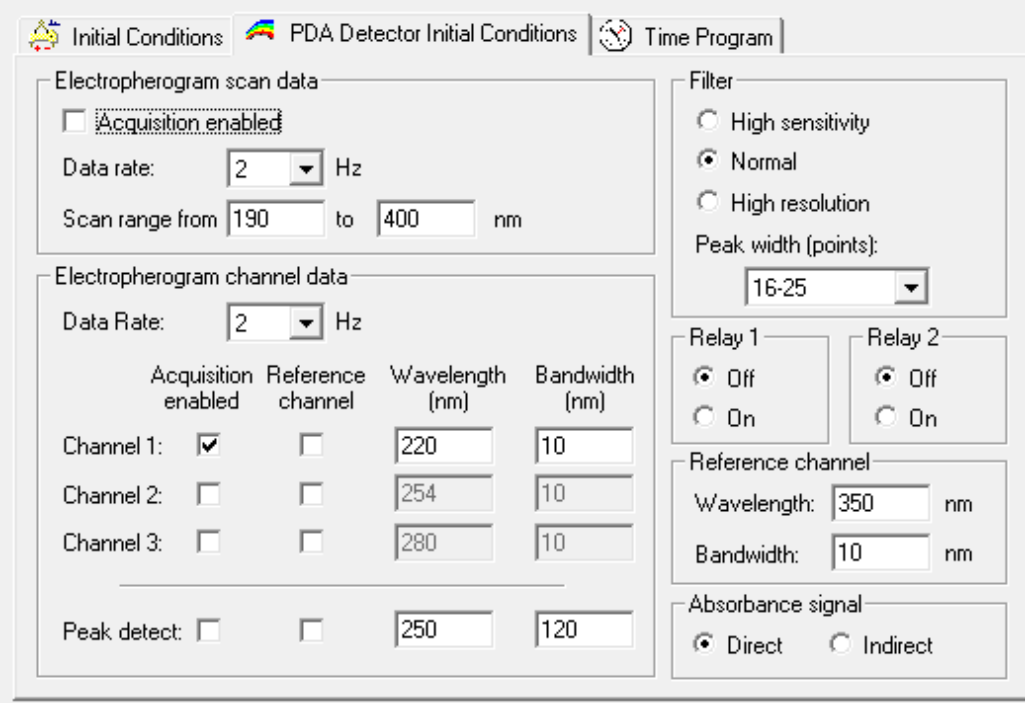
Initial Conditions

Figure B.1 Initial Conditions for All Methods



Detector Initial Conditions

Figure B.2 PDA Detector Initial Conditions for All Methods



Time Programs

The time programs are different for each method.

High-speed Method Time Programs

Figure B.3 Time Program for the IgG HS Conditioning - PA 800 plus Method

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	reverse	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	reverse	0.1 N HCl rinse to neutralize capillary surface
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	reverse	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	reverse	SDS-MW Gel Buffer rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, normal polarity, both	SDS-MW Gel Buffer for voltage equilibration
6								

Figure B.4 Time Program for the IgG HS Separation - PA 800 plus Method

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D	reverse, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E	reverse, In / Out vial inc 8	0.1 N HCl rinse to neutralize capillary surface - Automatic increment every 8 runs
3		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F	reverse, In / Out vial inc 8	Water rinse to remove the acid residue - Automatic increment every 8 runs
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B	reverse, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5		Wait		0.00 min	BI:A1	BO:A	In / Out vial inc 8	ddH2O, use to clean capillary tip - Automatic increment every 8 runs
6		Wait		0.00 min	BI:A4	BO:A	In / Out vial inc 8	ddH2O, use to clean capillary tip - Automatic increment every 8 runs
7		Inject - Voltage	5.0 KV	20.0 sec	BI:C1	SO:A	Override, normal polarity	Sample injection
8		Wait		0.00 min	BI:B4	BO:B	In / Out vial inc 8	ddH2O, use to avoid sample carry over - Automatic increment every 8 runs
9	0.00	Separate - Voltage	15.0 KV	15.00 min	BI:C1	BO:C	1.00 Min ramp, normal polarity, both, In /	SDS Gel for separation - Automatic increment every 8 runs
10	2.00	Autozero						
11								

Figure B.5 Time Program for the IgG HS Shutdown - PA 800 plus Method

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	10.00 min	BI:D1	BO:D	reverse	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E	reverse	0.1 N HCl rinse to neutralize capillary surface
3		Rinse - Pressure	50.0 psi	2.00 min	BI:F1	BO:F	reverse	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B	reverse	SDS Gel rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C	5.00 Min ramp, normal polarity, both	SDS Gel for voltage equilibration
6	10.00	Wait		0.00 min	BI:A1	BO:A		ddH2O, use for capillary dip to prevent capillary from drying
7	10.00	Lamp - Off						
8								

High-resolution Method Time Programs

Figure B.6 Time Program for the IgG HR Conditioning - PA 800 plus Method

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS -MW Gel Buffer rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS-MW Gel buffer voltage equilibration
6								

Figure B.7 Time Program for the IgG HR Separation - PA 800 plus Method

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 N HCl rinse to neutralize capillary surface silanol group - Automatic increment every 8 runs
3		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the acid residue - Automatic increment every 8 runs
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
6		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
7		Inject - Voltage	5.0 KV	20.0 sec	SI:A1	BO:C1	Override, reverse polarity	Sample injection
8		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	ddH2O, use for dipping to avoid sample carry over - Automatic increment every 8 runs
9	0.00	Separate - Voltage	15.0 KV	30.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
10	5.00	Autozero						
11								

Figure B.8 Time Program for the IgG HR Shutdown - PA 800 plus Method

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	50.0 psi	2.00 min	BI:F1	BO:F1	forward	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for separation
6	10.00	Wait		0.00 min	BI:A1	BO:A1		Water used for capillary dip to prevent capillary from drying
7	10.00	Lamp - Off						
8								

APPENDIX C

Using the Low pH SDS Sample Buffer

Note: SCIEX carries two different low pH sample buffers, the Low pH SDS Sample Buffer (Tris; pH 6.8) and the Low pH Phosphate SDS Sample Buffer (pH 6.5).

Some samples may be more stable in a sample buffer with a lower pH. To use the Low pH SDS Sample Buffer (pH 6.8), prepare the samples as described previously, but replace the SDS-MW Sample Buffer with the Low pH SDS Sample Buffer (pH 6.8).

Due to the increased ionic strength of low pH sample buffers, we recommend modifying the SDS-MW separation method by increasing the injection voltage or duration to avoid any signal loss. Adjust the separation time based on the samples to be analyzed. For example, for analysis of a Rituxan (rituximab) sample, change the separation time to 35 minutes.

Alternatively, use a pressure injection in the separation method. Start with the same SDS-MW separation method as for samples prepared with the SDS-MW Sample Buffer and edit the method as described in the following section.

Add a Pressure Injection to the SDS MW Separation Method

Use the following instructions to add a pressure injection and make other necessary changes to the separation method.

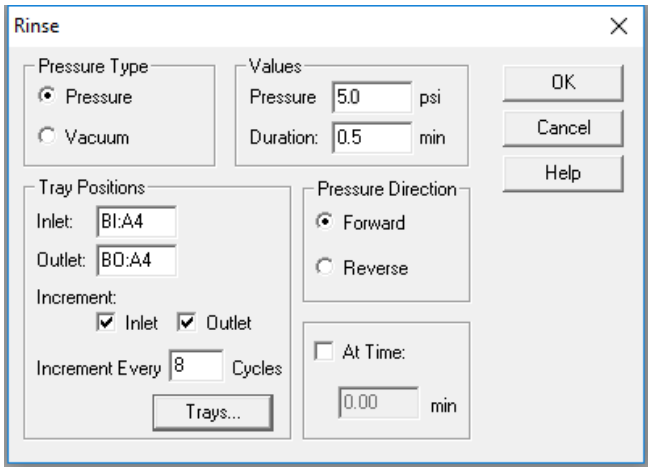
1. Open the SDS MW separation method in the 32 Karat™ Software.

   No changes to the Initial Conditions or the PDA Detector Initial Conditions are required.

2. Click the **Time Program** tab.

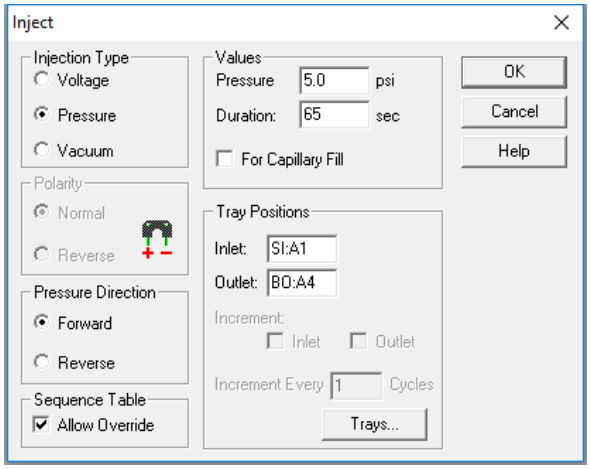
3. Add a **Rinse** event after row 5. Set the parameters as shown.

Figure C.1 Rinse Dialog



4 Edit the **Inject-Voltage** event to match the following figure.

Figure C.2 Inject Dialog



5 Adjust the duration of the **Separation-Voltage** event based on samples to be analyzed.

The time program should match the following figure.

Figure C.3 Separation Method Time Program after Editing (showing Rinse and Injection Pressure Events)

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 M NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 M HCl rinse to neutralize capillary surface - Automatic increment every 8 runs
3		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the residual acid - Automatic increment every 8 runs
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	Water dip to clean capillary tip - Automatic increment every 8 runs
6		Rinse - Pressure	5.0 psi	0.50 min	BI:A4	BO:A4	forward, In / Out vial inc 8	Introduce a water plug
7		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	Water dip to clean capillary tip - Automatic increment every 8 runs
8		Inject - Pressure	5.0 psi	65.0 sec	SI:A1	BO:A4	Override, forward	Sample injection
9		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	Water dip to prevent sample carry over - Automatic increment every 8 runs
10	0.00	Separate - Voltag	15.0 KV	35.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
11	5.00	Autozero						
12								

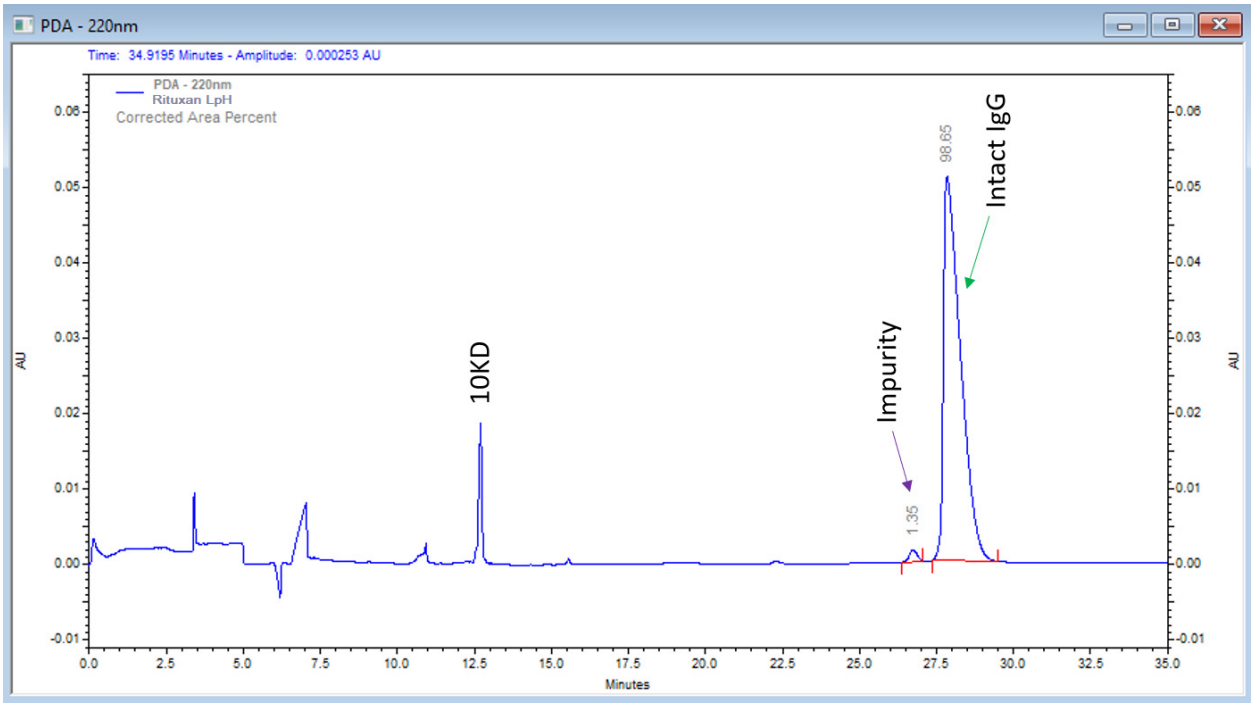
Apply

6 Save the method. If the method name is not changed, then no changes to the sequence are required.

Results Obtained Using the Low pH SDS Sample Buffer

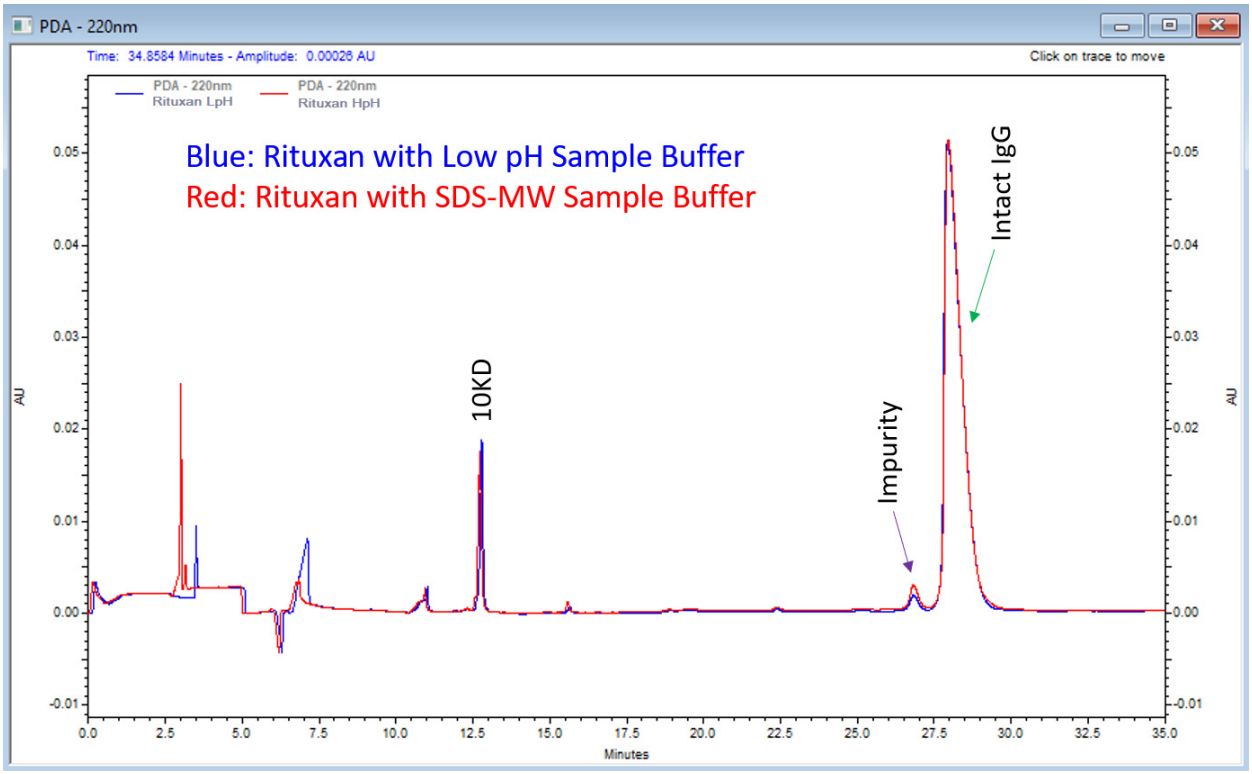
The following figure shows the results obtained with Rituxan (rituximab) and the Low pH SDS Sample Buffer. Refer to Figure C.4. Sample impurity was 1.35%.

Figure C.4 Rituxan (rituximab) Electropherogram Using the Low pH SDS Sample Buffer (Tris)



A comparison of results obtained with the SDS-MW Sample Buffer (red trace) and the Low pH SDS Sample Buffer (blue trace) with the Rituxan (rituximab) is shown in the following figure.

Figure C.5 Comparison of SDS-MW Sample Buffer and Low pH SDS Sample Buffer (Tris) for Rituxan (rituximab)



APPENDIX D

Using the Low pH Phosphate SDS Sample Buffer

Note: SCIEX carries two different low pH sample buffers, the Low pH SDS Sample Buffer (Tris; pH 6.8) and the Low pH Phosphate SDS Sample Buffer (pH 6.5).

About the Low pH Phosphate SDS Sample Buffer

The Low pH Phosphate SDS Sample Buffer is designed to meet specifications provided in the *Chinese Pharmacopeia* set forth by the Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China for capillary electrophoresis SDS separations.

In addition to specifying the buffer, the *Chinese Pharmacopeia* (2019-06-27) has recommendations for the separation method. Refer to *Chinese Pharmacopeia* 2020 Edition, Chapter 3127, 3127 单抗分子大小变 异体测定法 (CE-SDS法) or https://www.chp.org.cn/gjydw/swzp/5032.jhtml. The link was current at the time of publication.

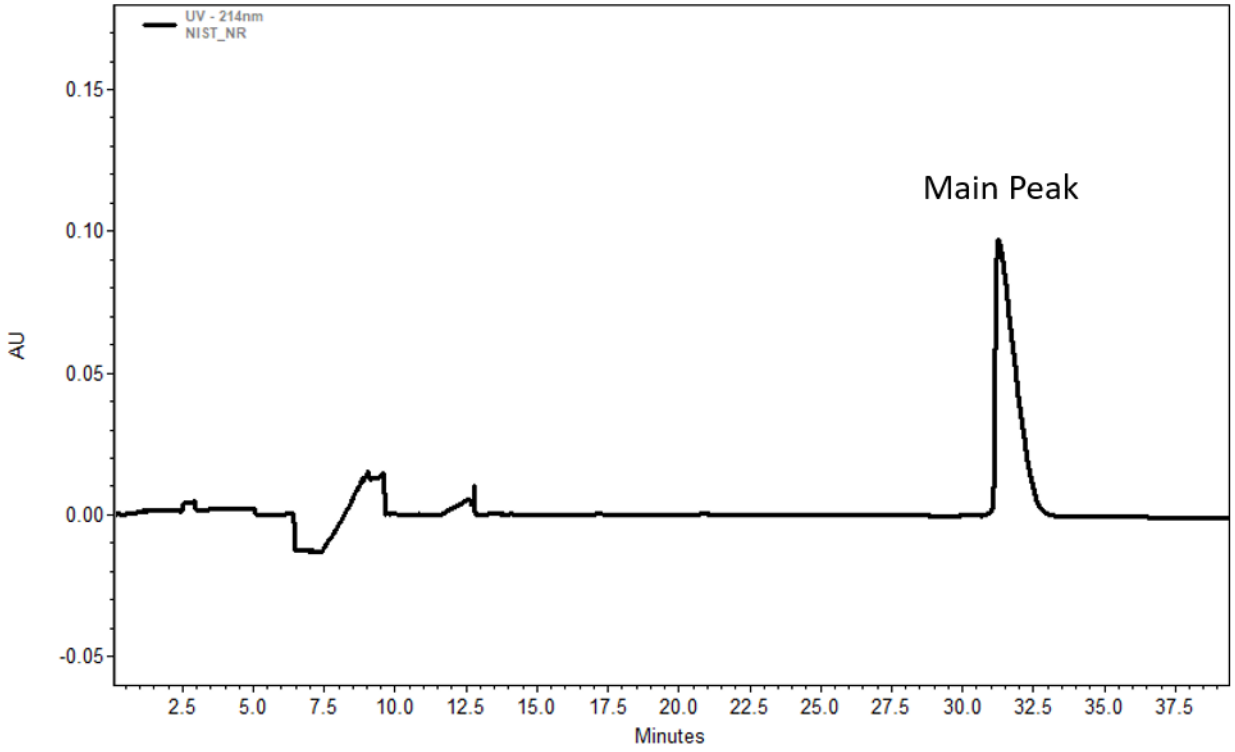
Note: The *Chinese Pharmacopeia* specifies a range for some assay parameters instead of a single value. For the results in the following figures, the median was used. Specifically, the sample incubation temperature was 70 °C and the sample storage and capillary temperatures were 20 °C.

Results Obtained Using the Low pH Phosphate SDS Sample Buffer

Typical Results Using Non-reducing Conditions

The following figure shows the results obtained with NIST mAb and the Low pH Phosphate SDS Sample Buffer following the *Chinese Pharmacopeia* method under non-reducing conditions.

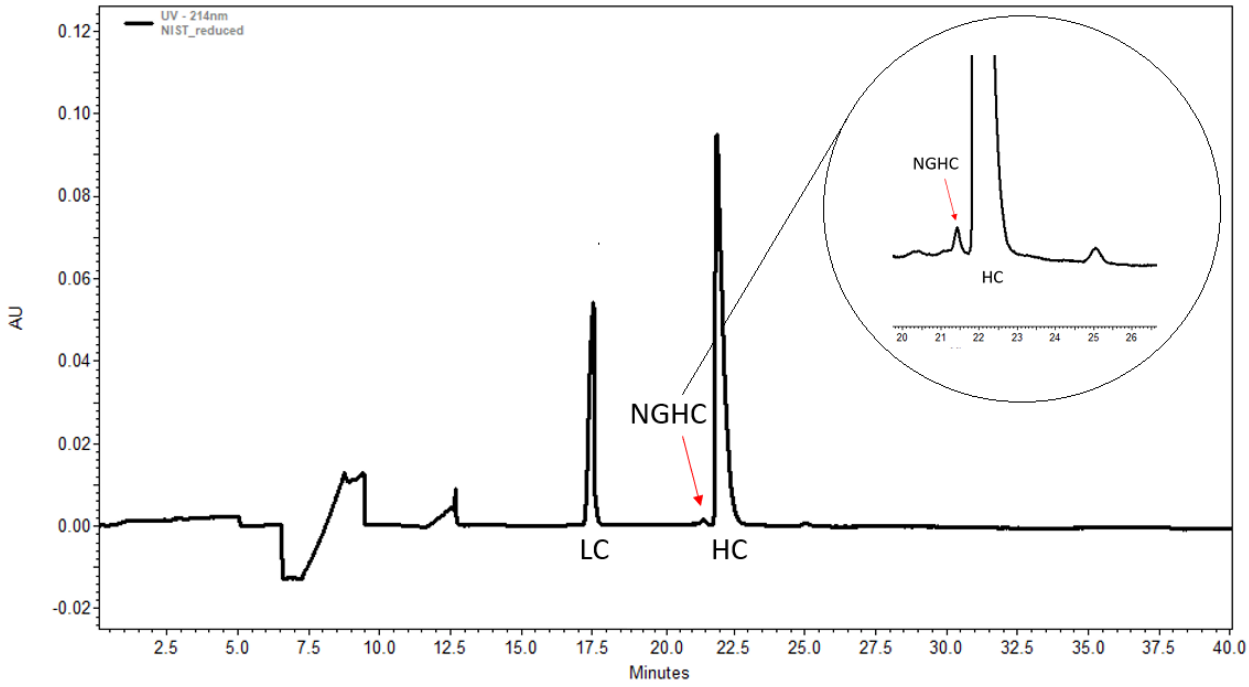
Figure D.1 Electropherogram of Non-reduced NIST mAb Using the Low pH Phosphate SDS Sample Buffer with the *Chinese Pharmacopeia* Method



Typical Results Using Reducing Conditions

The following figure shows the results obtained with NIST mAb and the Low pH Phosphate SDS Sample Buffer following the *Chinese Pharmacopeia* method under reducing conditions.

Figure D.2 Electropherogram of Reduced NIST mAb Using the Low pH Phosphate SDS Sample Buffer with the *Chinese Pharmacopeia* Method



- LC: light chain
- NGHC: non-glycosylated heavy chain
- HC: heavy chain


SCIEX
The Power of Precision

APPENDIX E

Run the Samples with the Waters Empower™ Software

This section gives instructions on data acquisition using the Empower™ Software. Refer to the Empower™ Software guides and help file for data analysis instructions.

Note: Calibrate the PDA detector before acquiring data. Refer to the *PA 800 Plus Empower™ Driver User Guide* for instructions.

Create the Instrument Methods

Note: If a low pH SDS sample buffer is used, the instrument methods might need adjustments to accommodate the increased ionic strength of the buffer. Refer to Using the Low pH SDS Sample Buffer.

Note: The validated instrument methods are included on the PA 800 Plus Empower™ Driver DVD. The methods can be imported instead of being creating manually. Refer to Import the Instrument Methods. If the methods are missing, then use the following instructions to create them.

Note: The following instrument methods are for the high-resolution separation. To perform a high-speed separation, refer to the instrument methods described in High-speed Instrument Methods.

Three instrument methods are required:

- IgG_CONDITIONING
- IgG_SEPARATION
- IgG_SHUTDOWN

Note: The values in the General and Detector tabs are the same for all of the methods.

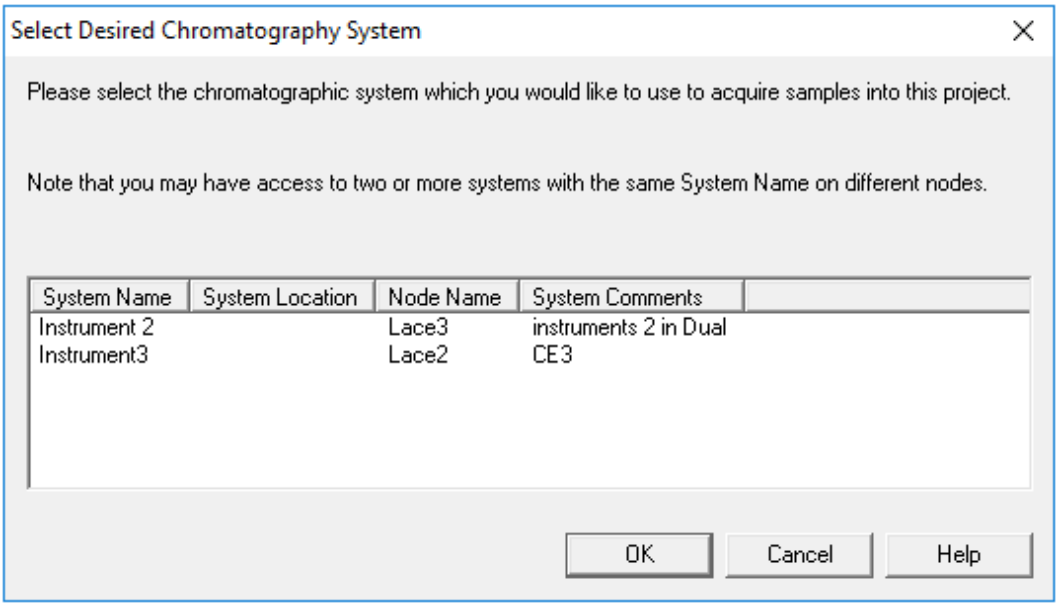
Note: Pressure values can be shown in millibar (mbar) or pounds per square inch (psi), depending on a registry setting for the Empower™ Software. The default unit is millibar. To change the units, refer to the *PA 800 Plus Empower™ Driver Release Notes*.

Note: The instrument methods that follow were validated using the SDS-MW Sample Buffer.

1 In the Empower™ Software Project window, click **File > New Method > Instrument Method**.

The Select Desired Chromatography System dialog opens.

Figure E.1 Select Desired Chromatography System Dialog

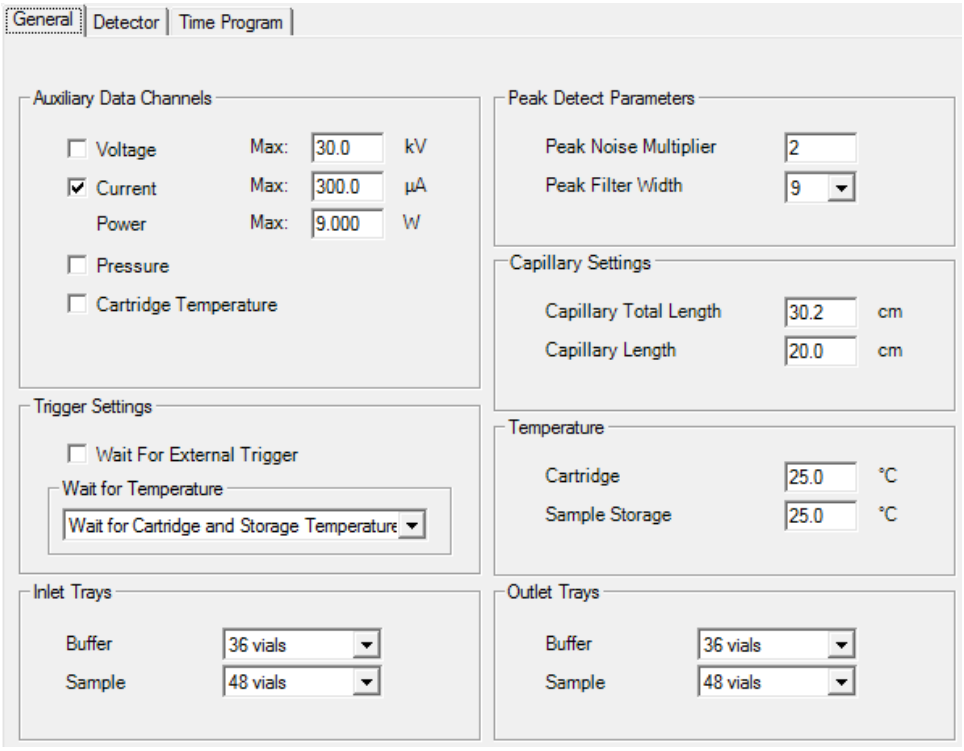


2 Click the system to be used and then click **OK**. Make sure that the instrument is configured with a PDA detector.

The Instrument Method Editor opens.

3 Set the parameters in the **General** tab.

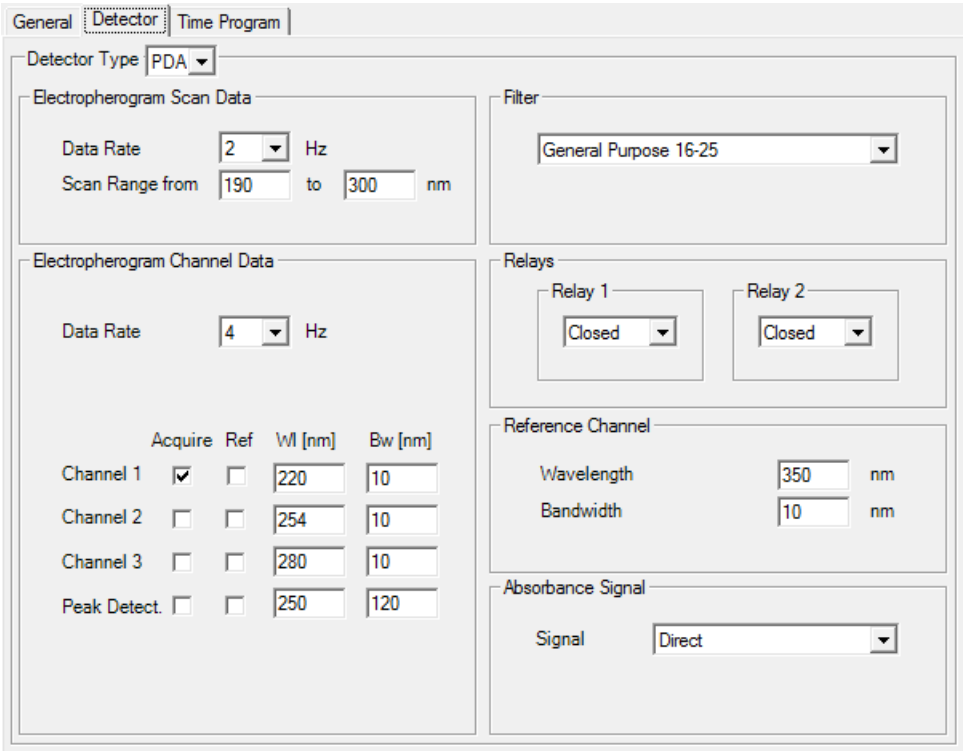
Figure E.2 General Parameters for the IgG_HR_CONDITIONING Instrument Method



4 Click the **Detector** tab, select **PDA** in the **Detector Type** list, and then set the parameters.

Note: For 3D data, in **Electropherogram Scan Data**, select **On** for **Data Rate**.

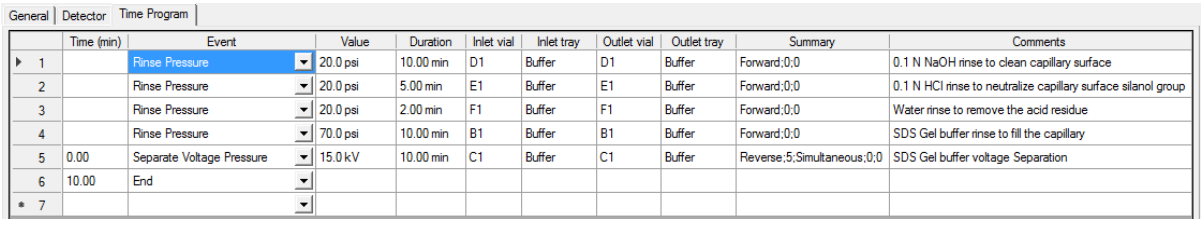
Figure E.3 Detector Parameters for the IgG_HR_CONDITIONING Instrument Method



5 Add the events in the following figure to the time program.

Note: For the pressure in the **Separate Voltage Pressure** event (step 5), type **20**.

Figure E.4 Time Program for the IgG_HR_CONDITIONING Instrument Method



	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	20.0 psi	10.00 min	D1	Buffer	D1	Buffer	Forward;0;0	0.1 N NaOH rinse to clean capillary surface
2		Rinse Pressure	20.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse Pressure	20.0 psi	2.00 min	F1	Buffer	F1	Buffer	Forward;0;0	Water rinse to remove the acid residue
4		Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Forward;0;0	SDS Gel buffer rinse to fill the capillary
5	0.00	Separate Voltage Pressure	15.0 kV	10.00 min	C1	Buffer	C1	Buffer	Reverse;5;Simultaneous;0;0	SDS Gel buffer voltage Separation
6	10.00	End								
* 7										

Note: If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1, 2, and 3), type **1379.0**.
- For the pressure in the **Rinse Pressure** event (step 4), type **4826.3**.
- For the pressure in the **Separate Voltage Pressure** event (step 5), type **1379.0**.

6 Save the instrument method.

a. Click **File > Save**. The Save current Instrument Method dialog opens.

b. Type **IgG_HR_CONDITIONING** in the **Name** field.

c. (Optional) Type information in the **Method Comments** field.

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

The instrument method is saved to the current project.

7 Create the separation instrument method.

a. Set the parameters on the **General** tab. Refer to Figure E.2.

b. Set the parameters on the **Detector** tab. Refer to Figure E.3.

c. Add the events in the following figure to the time program.

Note: For the pressure in the **Separate Voltage Pressure** event (step 9), type **20**.

Figure E.5 Time Program for the IgG_HR_SEPARATION Instrument Method

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	70.0 psi	3.00 min	D1	Buffer	D1	Buffer	Forward;8;8	0.1 N NaOH rinse to clean capillary surface
2		Rinse Pressure	70.0 psi	1.00 min	E1	Buffer	E1	Buffer	Forward;8;8	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse Pressure	70.0 psi	1.00 min	F1	Buffer	F1	Buffer	Forward;8;8	Water rinse to remove the acid residue
4		Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Forward;8;8	SDS Gel rinse to fill the capillary with SDS gel
5		Wait		0.00	A1	Buffer	A1	Buffer	8;8	Water. clean capillary tip
6		Wait		0.00	A4	Buffer	A4	Buffer	8;8	Water. clean capillary tip
7		Inject Voltage	5.0 kV	20.0 s	A0	Sample List	C1	Buffer	Reverse;0;0	inject sample
8		Wait	15.0 kV	0.00	B4	Buffer	B4	Buffer	8;8	water. use for dipping to avoid sample carry over
9	0.00	Separate Voltage Pressure	15.0 kV	30.00 min	C1	Buffer	C1	Buffer	Reverse;1;Simultaneous;8;8	SDS Gel Separation
10	5.00	Autozero								
11	30.00	Stop Data								
12	30.10	End								
* 13										

Note: If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1 through 4), type **4826.3**.
- For the pressure in the **Separate Voltage Pressure** event (step 9), type **1379.0**.

d. Save the method as "IgG_HR_SEPARATION".

8 Create the shutdown instrument method.

a. Set the parameters on the **General** tab. Refer to Figure E.2.

b. Set the parameters on the **Detector** tab. Refer to Figure E.3.

c. Add the events in the following figure to the time program.

Note: For the pressure in the **Separate Voltage Pressure** event (step 5), type **20**.

Figure E.6 Time Program for the IgG_HR_SHUTDOWN Instrument Method

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	70.0 psi	10.00 min	D1	Buffer	D1	Buffer	Forward;0;0	0.1 N NaOH rinse to clean capillary surface
2		Rinse Pressure	50.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse Pressure	50.0 psi	2.00 min	F1	Buffer	F1	Buffer	Forward;0;0	Water rinse to remove the acid residue
4		Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Forward;0;0	SDS Gel rinse to fill the capillary
5	0.00	Separate Voltage Pressure	15.0 kV	10.00 min	C1	Buffer	C1	Buffer	Reverse;5;Simultaneous;0;0	SDS Gel for Separation
6	10.00	Wait		0.00	A1	Buffer	A1	Buffer	0;0	STORAGE THE TIPS in water
7	10.00	Lamp Off								
8	10.00	End								
* 9										

Note: If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1 and 4), type **4826.3**.
- For the pressure in the **Rinse Pressure** events (steps 2 and 3), type **3447.4**.
- For the pressure in the **Separate Voltage Pressure** event (step 5), type **1379.0**.

d. Save the method as "IgG_HR_SHUTDOWN".

High-speed Instrument Methods

The General and Detector parameters for the high-speed methods are the same as for the high-resolution methods. However, the time programs are different.

Note: For the pressure in the **Separate Voltage Pressure** event (step 5), type **20**.

Figure E.7 Time Program for the IgG_HS_CONDITIONING Instrument Method

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	20.0 psi	10.00 min	D1	Buffer	D1	Buffer	Reverse;0;0	0.1 N NaOH rinse to clean capillary surface
2		Rinse Pressure	20.0 psi	5.00 min	E1	Buffer	E1	Buffer	Reverse;0;0	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse Pressure	20.0 psi	2.00 min	F1	Buffer	F1	Buffer	Reverse;0;0	Water rinse to remove the acid residue
4		Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Reverse;0;0	SDS Gel buffer rinse to fill the capillary
5	0.00	Separate Voltage Pressure	15.0 kV	10.00 min	C1	Buffer	C1	Buffer	Normal (+);5;Simultaneous;0;0	SDS Gel Buffer Separation
6	10.00	End								
** 7										

Note: If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1, 2, and 3), type **1379.0**.
- For the pressure in the **Rinse Pressure** event (step 4), type **4826.3**.
- For the pressure in the **Separate Voltage Pressure** event (step 5), type **1379.0**.

Note: For the pressure in the **Separate Voltage Pressure** event (step 9), type **20**.

Figure E.8 Time Program for the High-speed IgG_HS_SEPARATION Instrument Method

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	70.0 psi	3.00 min	D1	Buffer	D1	Buffer	Reverse;8;8	0.1 N NaOH rinse to clean capillary surface
2		Rinse Pressure	70.0 psi	1.00 min	E1	Buffer	E1	Buffer	Reverse;8;8	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse Pressure	70.0 psi	1.00 min	F1	Buffer	F1	Buffer	Reverse;8;8	Water rinse to remove the acid residue
4		Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Reverse;8;8	SDS Gel rinse to fill the capillary with SDS gel
5		Wait		0.00	A1	Buffer	A1	Buffer	8;8	Water clean capillary tip
6		Wait		0.00	A4	Buffer	A4	Buffer	8;8	Water clean capillary tip
7		Inject Voltage	5.0 kV	20.0 s	C1	Buffer	A0	Sample List	Normal (+);0;0	inject sample
8		Wait		0.00	B4	Buffer	B4	Buffer	0;0	
9	0.00	Separate Voltage Pressure	15.0 kV	15.00 min	C1	Buffer	C1	Buffer	Normal (+);1;Simultaneous;0;0	SDS Gel Separation
10	5.00	Autozero								
11	15.00	Stop Data								
12	15.00	End								
▸* 13										

Note: If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1 through 4), type **4826.3**.
- For the pressure in the **Separate Voltage Pressure** event (step 9), type **1379.0**.

Note: For the pressure in the **Separate Voltage Pressure** event (step 9), type **20**.

Figure E.9 Time Program for the High-speed IgG_HS_SHUTDOWN Instrument Method

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	70.0 psi	10.00 min	D1	Buffer	D1	Buffer	Reverse;0;0	0.1 N NaOH rinse to clean capillary surface
2		Rinse Pressure	50.0 psi	5.00 min	E1	Buffer	E1	Buffer	Reverse;0;0	0.1 N HCl rinse t neutralize capillary surface silanol group
3		Rinse Pressure	50.0 psi	2.00 min	F1	Buffer	F1	Buffer	Reverse;0;0	Water rinse to remove the acid residue
4		Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Reverse;0;0	SDS Gel rinse to fill the capillary
5	0.00	Separate Voltage Pressure	15.0 kV	10.00 min	A1	Buffer	A1	Buffer	Normal (+);5;Simultaneous;0;0	
6	10.00	Wait		0.00	A1	Buffer	A1	Buffer	0;0	Store the tips in water
7	10.00	Lamp Off								
8	10.00	End								
▸* 9										

Note: If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1 and 4), type **4826.3**.
- For the pressure in the **Rinse Pressure** events (steps 2 and 3), type **3447.4**.
- For the pressure in the **Separate Voltage Pressure** event (step 5), type **1379.0**.

Create the Method Sets

Three method sets are required:

- IgG Conditioning Method Set
- IgG Separation Method Set
- IgG Shutdown Method Set

Note: A method set can also include processing and report methods. To create those methods, refer to the documentation supplied with the Empower™ Software.

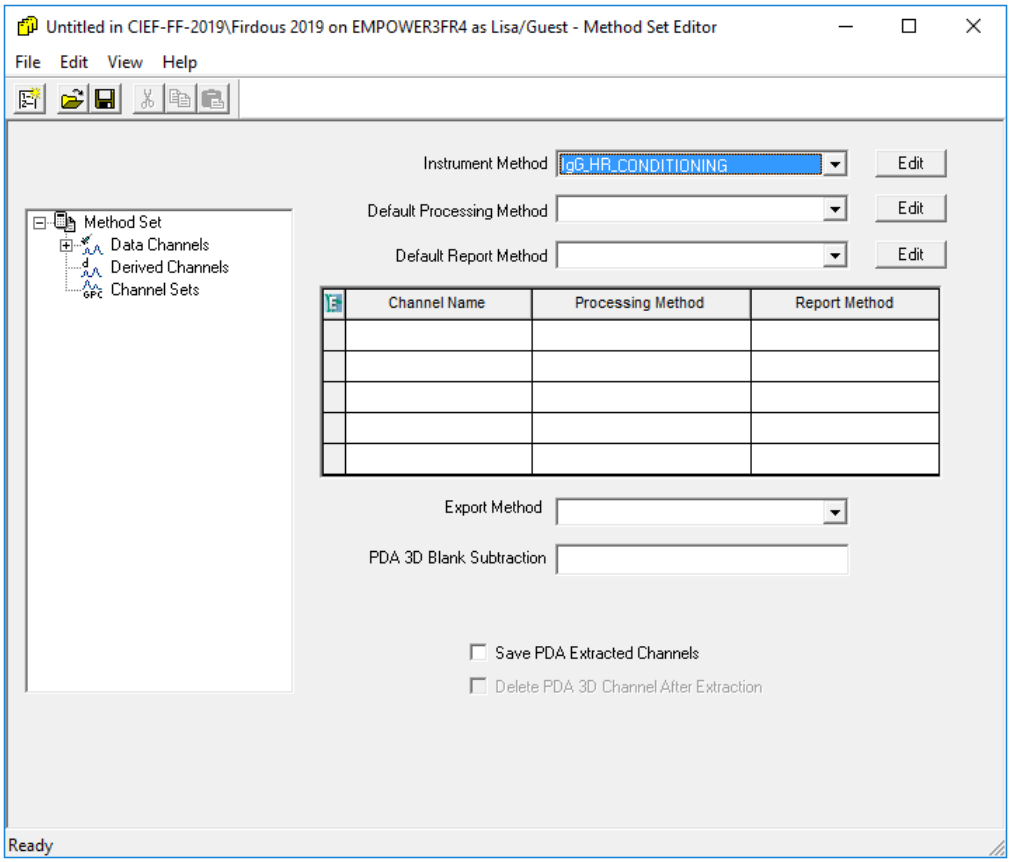
1 In the Empower™ Software Project window, click **File > New Method > Method Set**.

2 Click **No** in the message.

The Method Set Editor window opens.

3 In the **Instrument Method** list, click **IgG_HR_CONDITIONING**. Do not make any other changes.

Figure E.10 Method Set Editor Window



4 Save the method set.

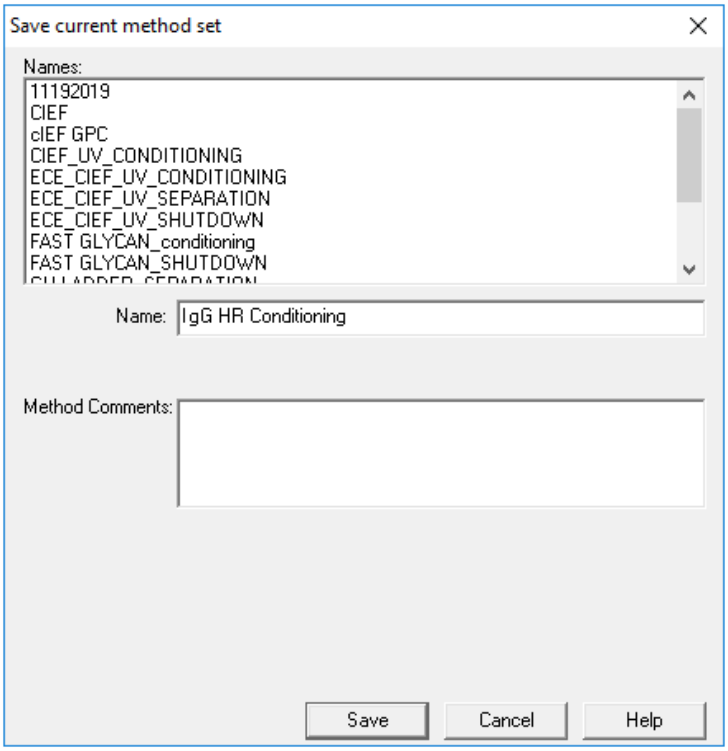
a. Click **File > Save** to open the Save current method set dialog.

b. Type **IgG HR Conditioning** in the **Name** field.

c. (Optional) Type information in the **Method Comments** field.

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

Figure E.11 Save current method set Dialog



The method set is saved to the current project.

5 Repeat the previous steps to create two more method sets.

a. Create the separation method set by selecting **IgG_HR_SEPARATION** in the **Instrument Method** list. Save the method set as “IgG Separation”.

b. Create the shutdown method set by selecting **IgG_HR_SHUTDOWN** in the **Instrument Method** list. Save the method set as “IgG Shutdown”.

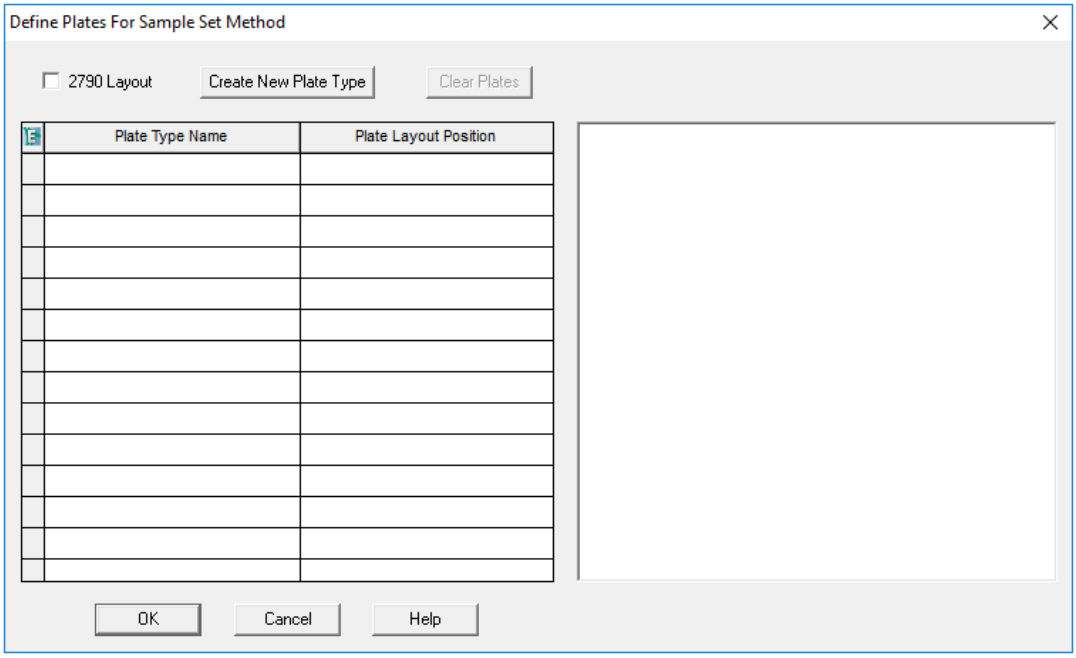
Configure the Software to Use Multiple Plates

The Empower™ Software is designed for chromatography systems that do not have buffer trays. To use the buffer trays, configure the Empower™ Software as follows.

1 In the Empower™ Software Run Samples window, click **Edit > Plates.**

The Define Plates for Sample Set Method dialog opens.

Figure E.12 Define Plates for Sample Set Method Dialog



Note: If the dialog does not look like the previous figure, clear the **2790 Layout** check box.

2 In the first row, set up the buffer inlet tray.

a. Click the **Plate Type Name** cell and then select **PA 800 Plus Buffer Tray.**

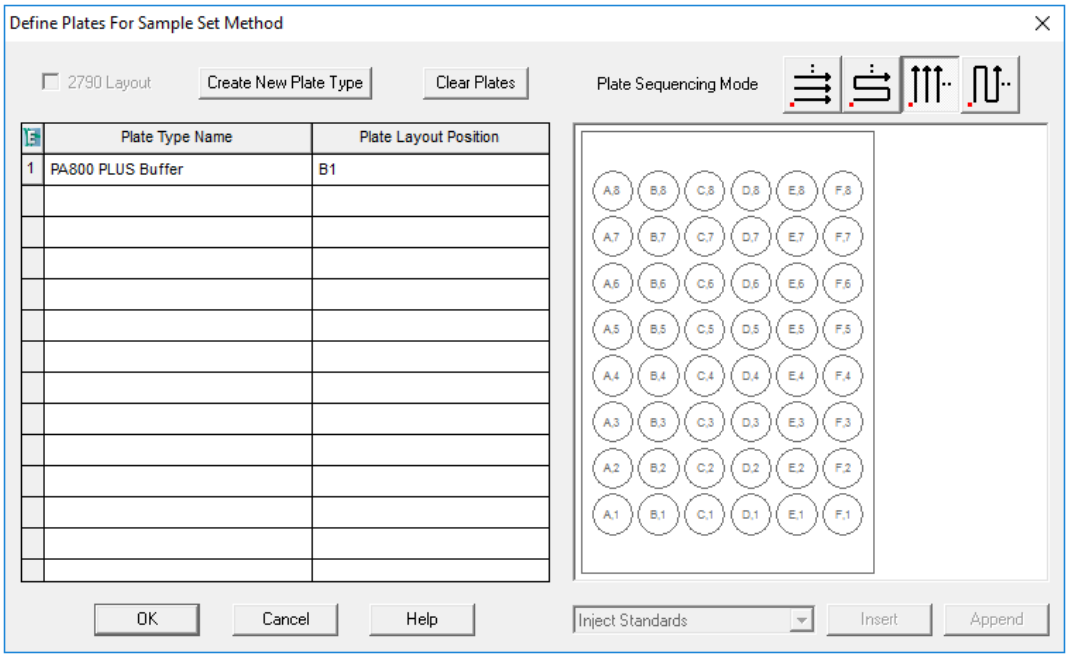
Note: If **PA 800 Plus Buffer Tray** is missing, then the buffer and sample trays might not have been defined. Refer to the *PA 800 Plus Empower™ Driver User Guide*.

The dialog updates with an image of the plate and buttons for the plate sequencing mode.

b. Click the **Plate Layout Position** cell and then type **BI**.

c. Click  (**Vertical Discontinuous Plate Sequencing Mode**) to indicate the order in which the vials are accessed during the run.

Figure E.13 After Defining the Buffer Inlet Plate



3 Repeat step 2 to set up the buffer outlet tray in the second row. Type **BO** for the **Plate Layout Position**.

4 In the third row, set up the sample inlet tray.

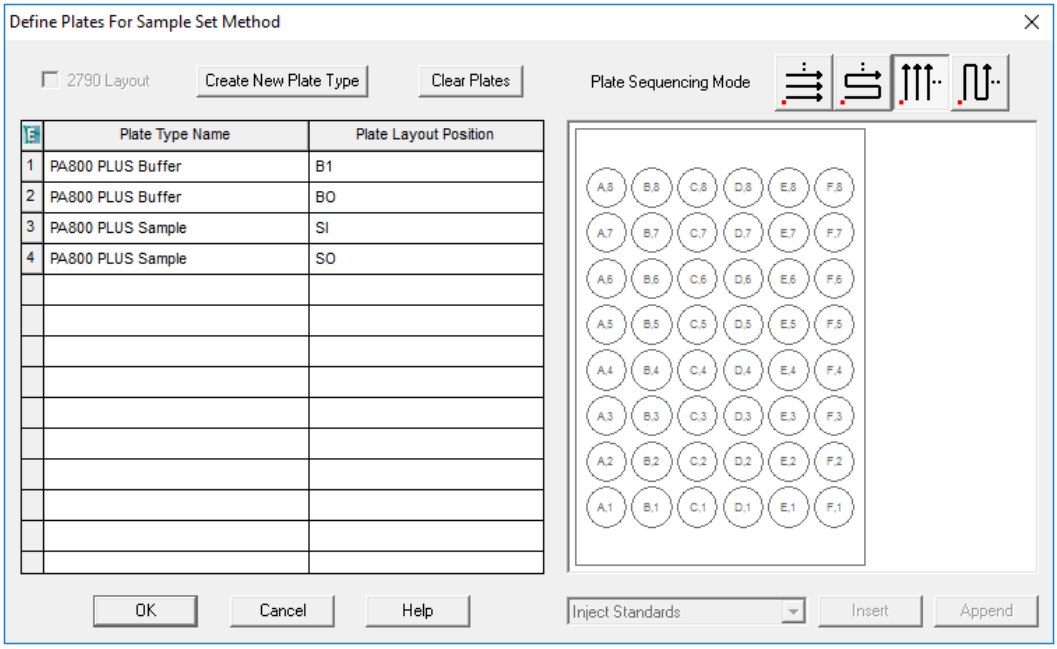
a. Click the **Plate Type Name** cell and then select the correct plate type, either **PA 800 Plus Sample Tray** or **PA 800 Plus 96 Well Sample Tray.**

b. Click the **Plate Layout Position** cell and then type **SI**.

c. Click  (**Vertical Discontinuous Plate Sequencing Mode**) to indicate the order in which the vials are accessed during the run.

5 Repeat step 4 to set up the sample outlet tray in the fourth row. Type **SO** for the **Plate Layout Position**.

Figure E.14 After Defining All Plate Types



6 Click **OK** to save the changes and close the dialog.

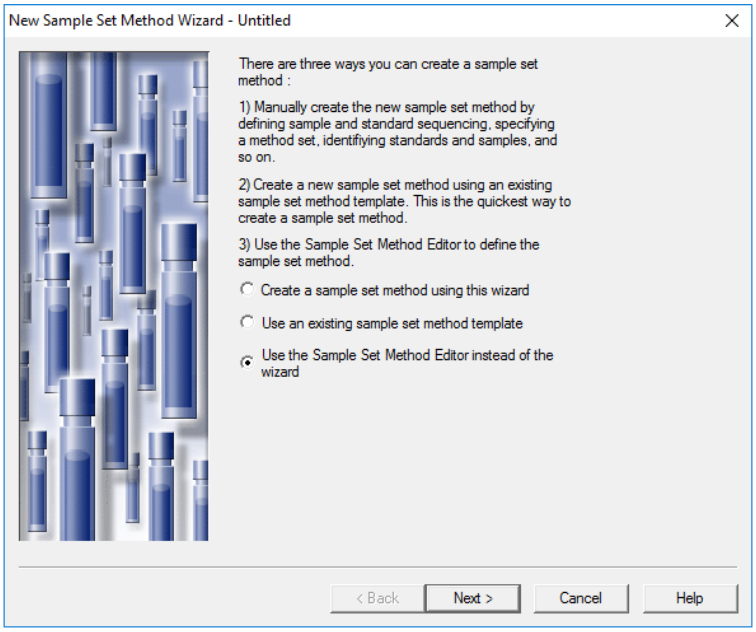
Create the Sample Set Method and Run the Samples

1 In the Empower™ Software Project window, click **File > New Method > Sample Set Method.**

The New Sample Set Method Wizard opens.

2 Click **Use the Sample Set Method Editor instead of the wizard** and then click **Next.**

Figure E.15 New Sample Set Method Wizard



The Sample Set Method Editor opens.

3 Set up the Sample Set Method.

a. In the first row, select **IgG HR Conditioning** in the **Method Set/Report or Export Method** cell.

b. For rows 2 through 17, select **IgG HR Separation** in the **Method Set/Report or Export Method** cell.

c. For row 18, select **IgG HR Shutdown** in the **Method Set/Report or Export Method** cell.

d. Add the required information for the samples. Refer to Table E.1. Use the default values for the other fields.

Table E.1 Required Fields for a Sample Set Method

Name	Description
Plate/Well	The position of the sample in the sample tray.
# of Injs	The number of times the sample is to be injected.
SampleName	The name of the sample.
Run Time (Minutes)	The duration of the run. **CAUTION: Possible Wrong Result. Make sure that the Run Time is greater than or equal to the duration of the time program in the instrument method. If the Run Time is shorter, the system stops the run before the time program is complete.**

The completed Sample Set Method is shown in the following figure.

Note: The **Level** and **Reference Level** columns are hidden in the following figure.

Figure E.16 Sample Set Method

	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Function	Method Set / Report or Export Method	Processing	Run Time (Minutes)
1	Bl:A,1	10.0	1		Conditioning	Inject Samples	IgG HR Conditioning	Normal	10.00
2	Sl:A,1	10.0	1	S0101	IgG STD	Inject Standards	IgG HR Separation	Normal	30.00
3	Sl:A,2	10.0	1	U0101	SAMPLE1	Inject Samples	IgG HR Separation	Normal	30.00
4	Sl:A,3	10.0	1	U0102	SAMPLE2	Inject Samples	IgG HR Separation	Normal	30.00
5	Sl:A,4	10.0	1	U0103	SAMPLE3	Inject Samples	IgG HR Separation	Normal	30.00
6	Sl:A,5	10.0	1	U0104	SAMPLE4	Inject Samples	IgG HR Separation	Normal	30.00
7	Sl:A,6	10.0	1	U0105	SAMPLE5	Inject Samples	IgG HR Separation	Normal	30.00
8	Sl:A,7	10.0	1	U0106	SAMPLE6	Inject Samples	IgG HR Separation	Normal	30.00
9	Sl:A,8	10.0	1	U0107	SAMPLE7	Inject Samples	IgG HR Separation	Normal	30.00
10	Sl:B,1	10.0	1	U0108	SAMPLE8	Inject Samples	IgG HR Separation	Normal	30.00
11	Sl:B,2	10.0	1	U0109	SAMPLE9	Inject Samples	IgG HR Separation	Normal	30.00
12	Sl:B,3	10.0	1	U0110	SAMPLE10	Inject Samples	IgG HR Separation	Normal	30.00
13	Sl:B,4	10.0	1	U0111	SAMPLE11	Inject Samples	IgG HR Separation	Normal	30.00
14	Sl:B,4	10.0	1	U0112	SAMPLE11	Inject Samples	IgG HR Separation	Normal	30.00
15	Sl:B,6	10.0	1	U0113	SAMPLE13	Inject Samples	IgG HR Separation	Normal	30.00
16	Sl:B,7	10.0	1	U0114	SAMPLE14	Inject Samples	IgG HR Separation	Normal	30.00
17	Sl:B,8	10.0	1	U0115	SAMPLE15	Inject Samples	IgG HR Separation	Normal	30.00
18	Sl:C,1	10.0	1	U0116	SAMPLE16	Inject Samples	IgG HR Separation	Normal	30.00
19	Sl:C,2	10.0	1	U0117	SAMPLE17	Inject Samples	IgG HR Separation	Normal	30.00
20	Sl:C,3	10.0	1	U0118	SAMPLE18	Inject Samples	IgG HR Separation	Normal	30.00
21	Sl:C,4	10.0	1	U0119	SAMPLE19	Inject Samples	IgG HR Separation	Normal	30.00
22	Sl:C,5	10.0	1	U0120	SAMPLE20	Inject Samples	IgG HR Separation	Normal	30.00
23	Sl:C,6	10.0	1	U0121	SAMPLE21	Inject Samples	IgG HR Separation	Normal	30.00
24	Sl:C,7	10.0	1	U0122	SAMPLE22	Inject Samples	IgG HR Separation	Normal	30.00
25	Sl:C,8	10.0	1	U0123	SAMPLE23	Inject Samples	IgG HR Separation	Normal	30.00
26	Bl:A,1	10.0	1		SAMPLE23	Inject Samples	IgG HR Shutdown	Normal	10.00

4 Save the Sample Set Method.

a. Click **File > Save**.

The Save current sample set method dialog opens.

b. Type **IgG Sample Set Method** in the **Name** field.

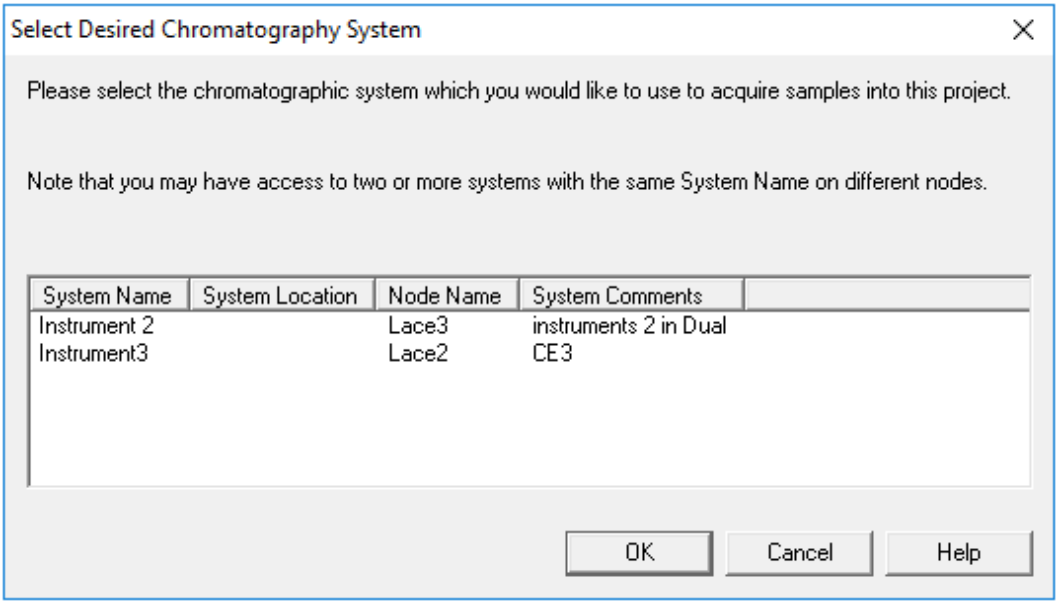
c. (Optional) Type information in the **Method Comments** field.

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

The method set is saved to the current project.

5 Click **Tools > Run Samples.**

Figure E.17 Select Desired Chromatography System Dialog



6 Click the system to be used and then click **OK.** Make sure that the instrument is configured with a PDA detector.

The Run Samples window opens.

7 Click  (**Load Sample Set**).

The Load Samples dialog opens.

8 Click **Load using a previously created sample set method** and then click **OK.**

Figure E.18 Load Samples Dialog

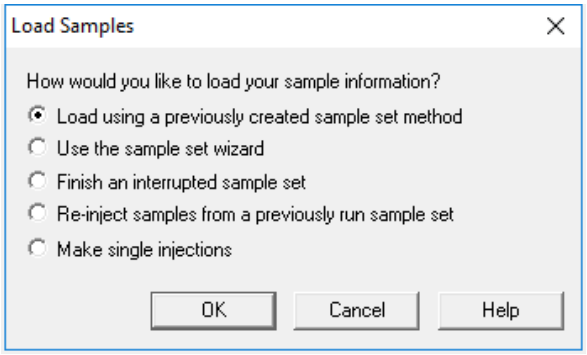
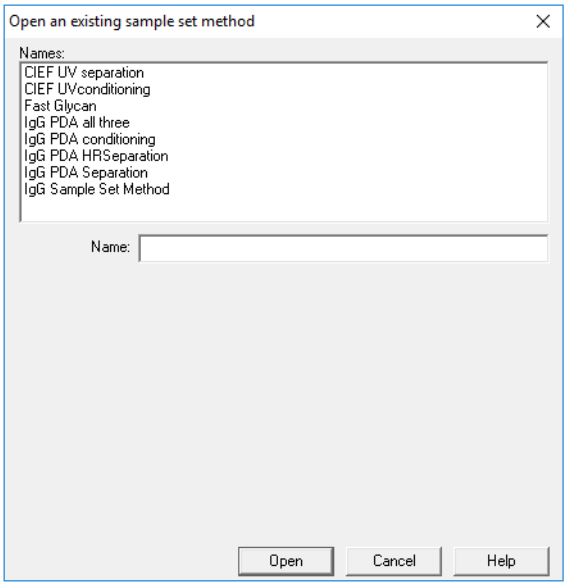


Figure E.19 Open an existing sample set method Dialog



9 Click **IgG Sample Set Method** in the list and then click **Open**.

The sample set method opens in the Samples tab.

10 In the Empower™ Software Project window, click (**Start**). Data acquisition starts.

During the run, the text in the row in the Sample Set Method window for the sample being acquired appears red.

11 During the run the following actions are available:

- (Optional) Click (**Stop**) to halt data acquisition.
- View the voltage and current data.

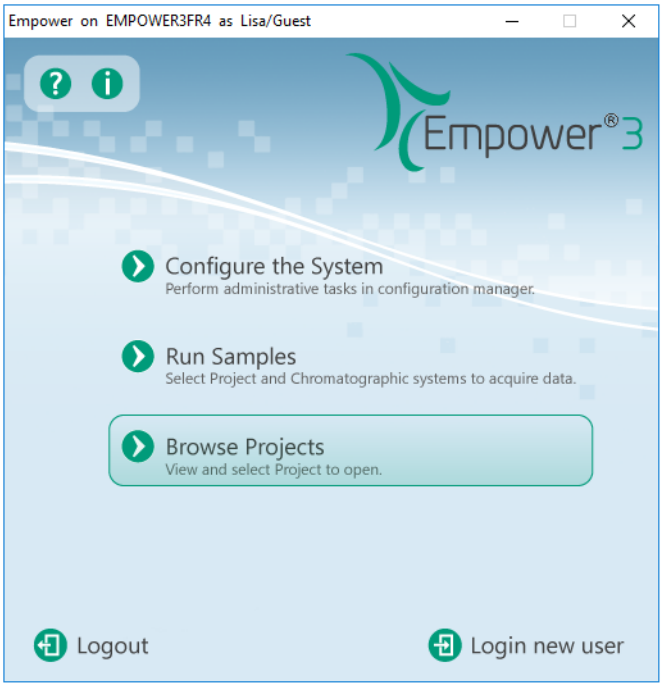
When the run ends, the text in all rows in the Sample Set Method window appears red.

Import the Instrument Methods

1 Open the **Methods** folder on the PA 800 Plus Empower™ Driver DVD.

2 In the Empower™ Software Pro Interface window, click **Browse Projects**, click the project of interest, and then click **OK**.

Figure E.20 Empower™ Software Pro Interface Window



The Project window opens.

3 Click the **Methods** tab.

4 On the Windows desktop, click each min file in the **Methods** folder and then drag it to the Project window.

The instrument method is added to the project and can be edited and added to a method set like any other method.


SCIEX
The Power of Precision

Revision History

Initial Issue, A51967AA, April 2009
32 Karat™ Software version 9.1
PA 800 *plus* Software version 1.1
PA 800 *plus* Firmware version 9.0

First Revision, A51967AB, December 2009
Revised corporate address.

Second Revision, A51967AC, February 2011
32 Karat™ Software version 9.1 patch
PA 800 *plus* Software version 1.1 patch
PA 800 *plus* Firmware version 9.2
Numerous syntax and grammatical edits

Third Revision, A51967AD, January 2014
Dimension & instruction edit

Fourth Revision, RUO-IDV-05-6935-A, April 2018
Rebranded. Applied new template. Legal content updated. Safety chapter removed and a reference to the safety content found in the System Overview Guide added. Replaced instructions for creating methods with instructions for using the PA 800 Plus Software. Added Hazardous Substance Information appendix.

Fifth Revision, RUO-IDV-05-6935-B, April 2020
Applied new template. Updated legal content. Replaced title Introduction with Overview. Added Required Detector and Required Cartridge or Capillary. Updated Methods and Sequences. Replaced title Capillary Cleaning and Storage with Store the Cartridge and included subtopics. Added Methods appendix. Added Run the Samples with the Waters Empower™ Software appendix. Added Contact Us.

Sixth Revision, RUO-IDV-05-6935-C, July 2020
Updated Legal Content. Updated Introduction. Updated Appendix A, Hazardous Substance Information. Added Appendix C, Low pH SDS Sample Buffer, including sample preparation instructions. Added Appendix D, Low pH Phosphate SDS Sample Buffer. Updated Contact Us.

This guide applies to the latest software and firmware listed above, and any higher subsequent versions. When a subsequent software or firmware version affects the information in this guide, a new issue will be released to the SCIEX website. For updates, go to sciex.com *and download the latest version of the guide.*


SCIEX
The Power of Precision

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™

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.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.