IgG Purity and Heterogeneity Assay Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

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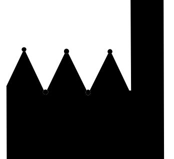

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IgG Purity and Heterogeneity Assay Kit

Prior to using the system, refer to the *PA 800 Plus Pharmaceutical Analysis System Overview Guide* for detailed information on the safe use and operation of the system.

Safety

Refer to the Safety Data Sheets (SDS), available at sciex.com/tech-regulatory, regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

Introduction

The PA 800 Plus IgG Purity and Heterogeneity Assay Kit includes methods to resolve both reduced and non-reduced immunoglobulins by size, and to quantify the heterogeneity and impurities which may exist in an IgG preparation. The methodology involves heat denaturing of a specified concentration of protein in the presence of SDS. Once denatured, the sample is separated by size in a capillary containing a replaceable SDS polymer matrix, which 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 (for example, 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.

NOTE The PA 800 series system must be equipped with a photodiode array (PDA) detector to perform this assay.

Intended Use

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

Equipment and Materials Required

Table 1 Kit Contents (PN A10663)

Component	Quantity	Reorder Part Number
Capillary, 50 µm I.D. bare-fused silica	2	N/A
SDS-MW Gel Buffer - proprietary formulation, pH 8, 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
Acidic Wash Solution, 0.1 M HCl	100 mL	N/A
Basic Wash Solution, 0.1 M NaOH	100 mL	N/A

Table 2 Additional Supplies from SCIEX

Component	Quantity	Part Number
Capillary, 50 µm I.D. bare-fused silica	1 box	N/A
Universal vials	100	A62251
Universal vial caps - blue	100	A62250
Micro vials, 200 µL	50	144709

Table 3 Additional Required Supplies

Component	Vendor	Part Number
2-mercaptoethanol	Sigma-Aldrich	M7154
Iodoacetamide	Sigma-Aldrich	I-1149
Microcon-30 kDa centrifugal filter unit with Ultracel-30 membrane	Millipore	MRCF0R030

Customer-Supplied Equipment and Supplies

- Pipettors and appropriate tips
- Vortex mixer
- Microcentrifuge
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2 µm filter and with resistance greater than 18 MΩ)
- Water bath or heat block (37 °C to 100 °C)
- Analytical balance
- Centrifugal vacuum evaporator
- Powder-free gloves (neoprene or nitrile recommended)
- Safety glasses
- Laboratory coat
- Parafilm
- Spatula
- Nonidet NP-40 non ionic detergent

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 promptly at –20 °C.
- Store the capillary, sample buffer, SDS-MW gel buffer, acidic wash solution (0.1 M HCl), and basic wash solution (0.1 M NaOH) at room temperature.

If precipitate is present in the SDS-MW gel Buffer or SDS-MW sample buffer, stir before use until the precipitate is fully dissolved. Before starting a CE separation, bring the SDS-MW gel buffer and the SDS-MW sample buffer to room temperature for a minimum of four hours.

Prepare the PA 800 Plus System

Before proceeding, you must understand the following procedures as described in the *PA 800 Plus System Maintenance Guide*:

- How to replace the capillary cartridge
- How to install the PDA detector
- How to calibrate the PDA detector

For instructions on loading and unloading trays, refer to the *PA 800 Plus System Overview Guide*.

Install the PDA Detection Module

1. Turn off the PA 800 Plus instrument and install the PDA detection module.
2. Turn on the instrument and permit the UV lamp to warm up for at least 30 minutes.

Clean the Interface

Carefully clean the system electrodes, capillary ends, opening levers, and interface block as described in the *PA 800 Plus System Maintenance Guide*, either once a day or after the finish of the sequence. The SDS-MW gel buffer is very viscous and will accumulate on the capillary ends, electrodes, interface block, and opening levers if regular and thorough cleaning is not performed. Gel accumulation might cause broken capillaries, bent electrodes, vial jams, and missed injections.

Insert the Cartridge and Calibrate the PDA Detector

Insert the cartridge into the system. Close the front panel and calibrate the PDA detector. This procedure should be employed daily or any time the cartridge is replaced.

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 mix thoroughly.
5. Centrifuge for 1 minute at 300 *g*.
6. Seal the vial cap with parafilm and heat at 70 °C for 10 minutes.

7. Put the vial in a room-temperature water bath to cool for at least 3 minutes.

8. Transfer 100 µL of the prepared sample to a micro vial, put the micro vial into a universal vial, and cap the universal vial.

Prepare the IgG Control Standard (Non-Reducing)

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 thoroughly until dissolved, and then store in the dark.

Prepare the IgG Non-reduced 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 mix throughly.

5. Centrifuge for 1 minute at 300 *g*.

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

7. Put the vial in a room-temperature water bath to cool for at least 3 minutes.

8. Transfer 100 µl of the prepared sample to a micro vial. Put the micro vial into a universal vial, and 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 the buffer of the IgG sample must be exchanged with SDS-MW sample buffer by using the Microcon-30 kDa centrifugal filter unit. Follow the instructions provided in *Perform a Buffer Exchange for the IgG Sample*.

Prepare the IgG Reduced 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 vial tightly and then mix thoroughly.

6. Centrifuge for 1 minute at 300 *g*.

7. Seal the tube with parafilm and heat at 70 °C for 10 minutes.

8. Put the vial in a room-temperature water bath to cool for at least 3 minutes and then transfer it to the sample vial.

9 Transfer 100 µL of the prepared sample to a micro vial. Put the micro vial inside a universal vial and cap the universal vial.

Perform a Buffer Exchange for the IgG Sample

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, low signal and peak tailing is likely to occur. Exchange the sample buffer with a Microcon-30 kDa centrifugal filter unit using the following procedure:

1. Add 1 mL of IgG sample to a Microcon-30 kDa centrifugal filter unit.
2. Centrifuge for 15 minutes at 4000 *g*.
3. Add 2 mL of SDS-MW sample buffer, then centrifuge for 25 minutes at 4000 *g*.
4. Insert the Microcon-30 kDa centrifugal filter unit into a new vial and then centrifuge for 3 minutes at 1000 *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 IgG Non-reduced 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 may 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 into 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 internal standard to the tube.
4. Inside a fume hood, add 5 µL of the 250 mM IAM solution to the sample tube.

5. Cap the vial tightly and mix thoroughly.
6. Centrifuge for 1 minute at 300 *g*.
7. Seal the tube with parafilm and heat the mixture in a water bath at 70 °C for 10 minutes.
8. Put the tube in a room-temperature water bath to cool for at least 3 minutes.
9. Transfer 100 µL of the prepared sample to a 200 µL micro vial and spin down the contents to remove any air bubbles. Put the micro vial inside a universal vial and cap the universal vial.

Set Up the System

Methods and Sequences

The methods, sequences, and data files are installed on the PA 800 Plus controller. They are not available for download.

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

- **IgG HR Conditioning - PA 800 plus.met** — To condition the capillary at the start of each day.
- **IgG HR Separation - PA 800 plus.met** — To perform an IgG separation.
- **IgG HR Shutdown - PA 800 plus.met** — For shutting down at the end of a sequence: to rinse the capillary for storage and to turn off the UV lamp.
- **IgG HS Conditioning - PA 800 plus.met** — To condition the capillary at the start of each day.
- **IgG HS Separation - PA 800 plus.met** — To perform an IgG separation.
- **IgG HS Shutdown - PA 800 plus.met** — For shutting down at the end of a sequence: to rinse the capillary for storage and to turn off the UV lamp.

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

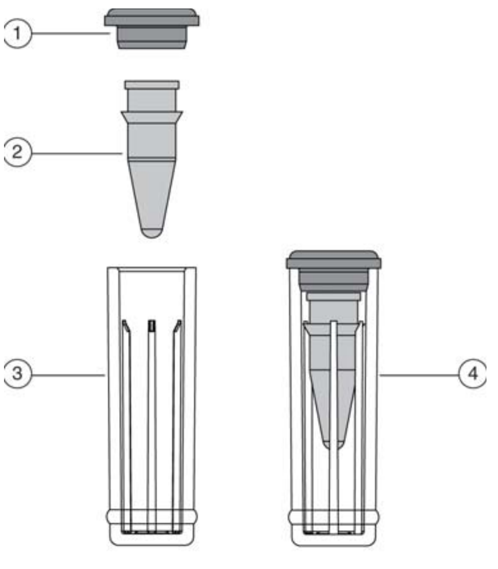
- **IgG HR - 24 samples - PA 800 plus.seq**
- **IgG HR - PA 800 plus.seq**
- **IgG HS - PA 800 plus.seq**

Prepare the Sample Vials

Before putting the 200 µL sample vials (or micro vials) into the universal vials, make sure that no bubbles are at the bottom of the vials. If bubbles exist, centrifuge the micro vials for 2 minutes at 1000 *g* and repeat if necessary. Put a blue cap on the universal vial and make sure it is secure. Refer to Figure 1.

Put the universal vials into the 48-position inlet sample tray from positions A1 through C8.

Figure 1 Sample Vial Setup



1. Universal Cap
2. Micro Vial
3. Universal Vial
4. Micro Vial inside Universal Vial

Prepare and Load the Buffer Vials

A sequence table with high-resolution (HR) methods is provided in the software: **IgG HR - PA 800 plus.seq**. This sequence can run up to 24 samples where sample number 1 is (always) the IgG control standard.

DANGER! Toxic Chemical Hazard. Read the *Safety Data Sheets* for 0.1 M NaOH Solution, 0.1 M NaOH, and SDS-MW Gel Buffer before use.

Refer to APPENDIX A for additional information.

Prepare the Reagent Vials

Fill the appropriate number of reagent vials with the SDS-MW gel buffer, 0.1 M NaOH solution, 0.1 M HCl solution, and DDI water according to the buffer tray maps (Figure 3 and Figure 4).

The number of reagent vials depends on the number of method cycles. The methods have been developed to automatically advance the reagent vials after eight cycles, providing a fresh set of buffers every eight cycles. The buffer tray maps are designed for use with the high-resolution methods, which introduce the sample from the left-side tray.

1. Fill the gel rinse (Gel-R) vials with 1.2 mL of SDS-MW gel buffer.

2. Fill the gel separation (Gel-S) vials with 1.1 mL of SDS-MW gel buffer.

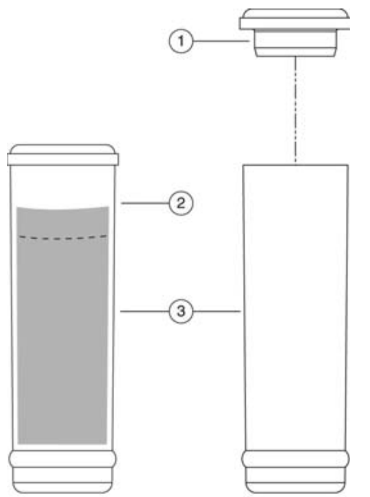
3. Fill the water (H_2O) vials with 1.5 mL of DDI water.

4. Fill the NaOH and HCl vials with 1.5 mL of NaOH and HCl, respectively.

5. Fill the waste vials with 1.0 mL of DDI water.

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 2 Universal Vials and Caps



1. Universal Vial Cap
2. Maximum Fill Level
3. Universal Vials

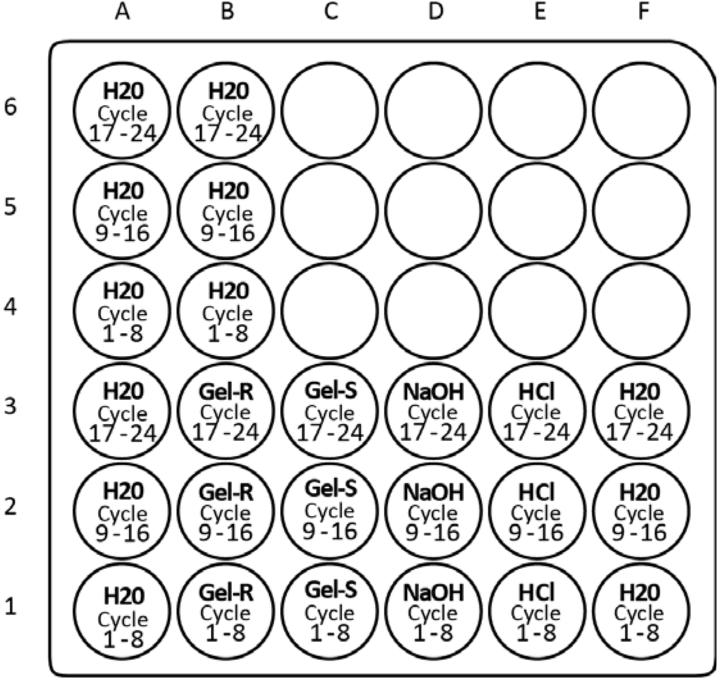
NOTE Carefully fill the buffer vials with SDS-MW gel buffer without producing bubbles and use the recommended volume. If the volume is too low (< ½ of vial volume), the capillary and electrode might not be able to dip into the SDS-MW gel buffer during the separation. If the volume is too high, the SDS-MW gel buffer may accumulate on the capillary ends and electrodes, causing various modes of system failure.

6 Cap the universal vials.

IMPORTANT In this application, all vials and caps are designed for a maximum of eight runs each. Do not reuse the caps. They can be contaminated with dried gel and other chemicals.

7 Load the reagent vials into the inlet and outlet 6x6 buffer trays as shown in Figure 3 and Figure 4.

Figure 3 Inlet Buffer Tray Map



1. H2O - DDI Water	**4.** NaOH
2. Gel-R - SDS-MW Gel Rinse	**5.** HCl
3. Gel-S - SDS-MW Gel Separation	

A1 to A6: DDI H_2O, use for dip step to clean capillary tip, 1.5 mL

B4 to B6: DDI H_2O, use for dip step to clean capillary tip, 1.5 mL

B1 to B3: SDS-MW gel buffer to rinse/fill capillary prior each cycle, 1.2 mL

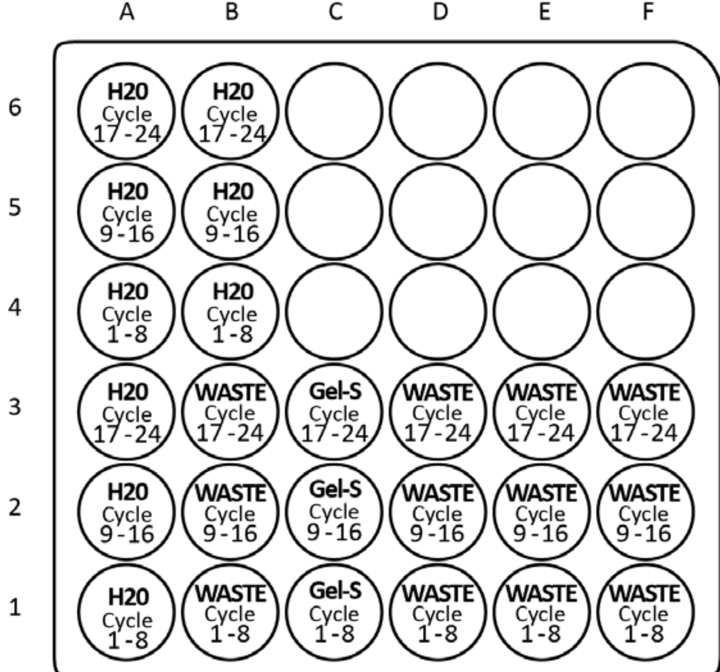
C1 to C3: SDS-MW gel buffer for separation, 1.1 mL

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

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

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

Figure 4 Outlet Buffer Tray Map



1. H2O - DDI Water **2.** WASTE	**3.** Gel-S - Gel Separation

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

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

B1 to B3: Waste vial for SDS-MW gel buffer rinse, 1.0 mL of DDI water

C1 to C3: SDS-MW gel buffer for separation, 1.1 mL

D1 to D3: Waste vial for 0.1 M NaOH rinse, 1.0 ml of DDI water

E1 to E3: Waste vial for 0.1 M HCl rinse, 1.0 ml of DDI water

F1 to F3: Waste vial for DDI H_2O rinse, 1.0 ml of DDI water

8 Load the trays into the PA 800 Plus system.

Run the Assay

Create the Sequence and Start the Run

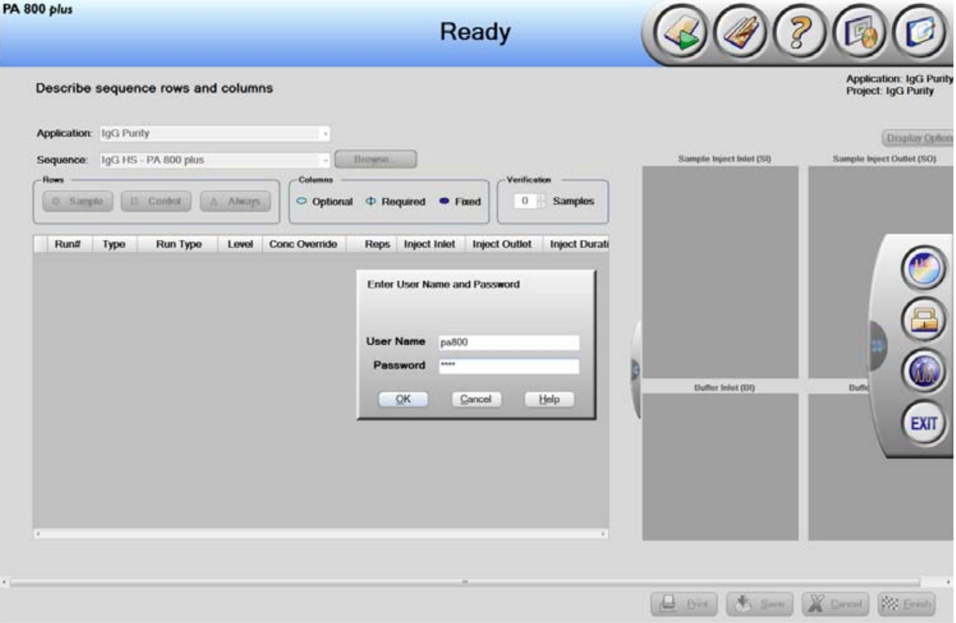
1 Double-click the PA 800 Plus software icon on the desktop.

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 HiSpeed - PA 800 plus.seq**.

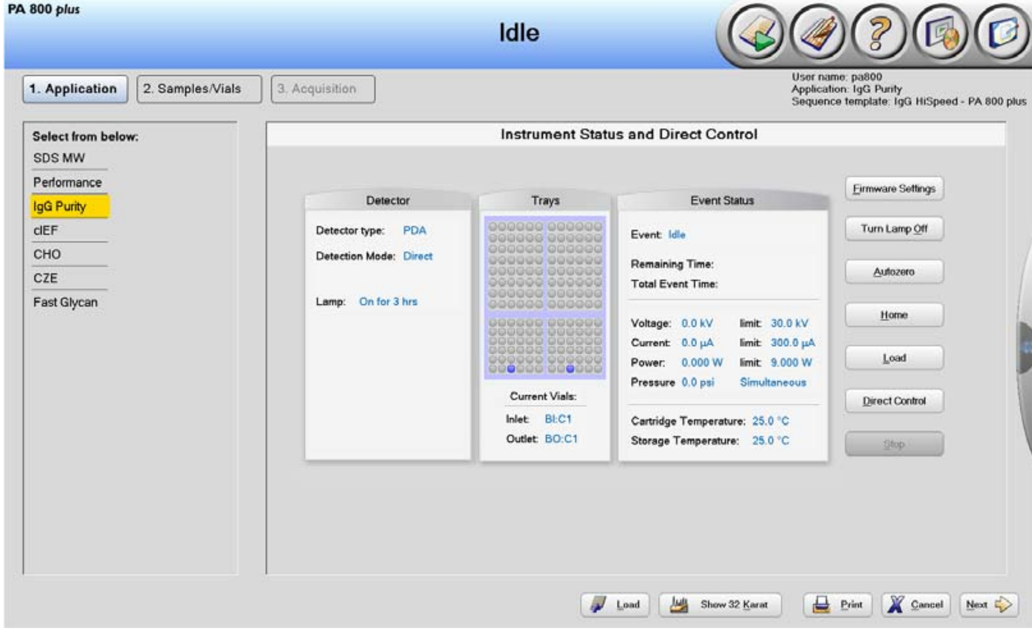
If system administration is enabled, type the user name and the password when prompted, and then click **OK** (Figure 5). The default user name is **pa800**, and the default password is **plus**.

Figure 5 User Name and Password Entry



The Instrument Status and Direct Control window opens (Figure 6).

Figure 6 Instrument Status and Direct Control Window



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

5 Select **IgG HiSpeed - PA 800 plus.seq** 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.

NOTE The Describe function can be accessed from the PA 800 Plus window, the application window, or from the Samples/Vials window by clicking the Describe icon.

Use the Describe function to customize the sequence stable and edit the number of samples that can be run in the sequence. The Describe function can set the row types as controls for system suitability standards and blanks, samples for unknowns, and when preparing for capillary conditioning and shutdown runs.

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

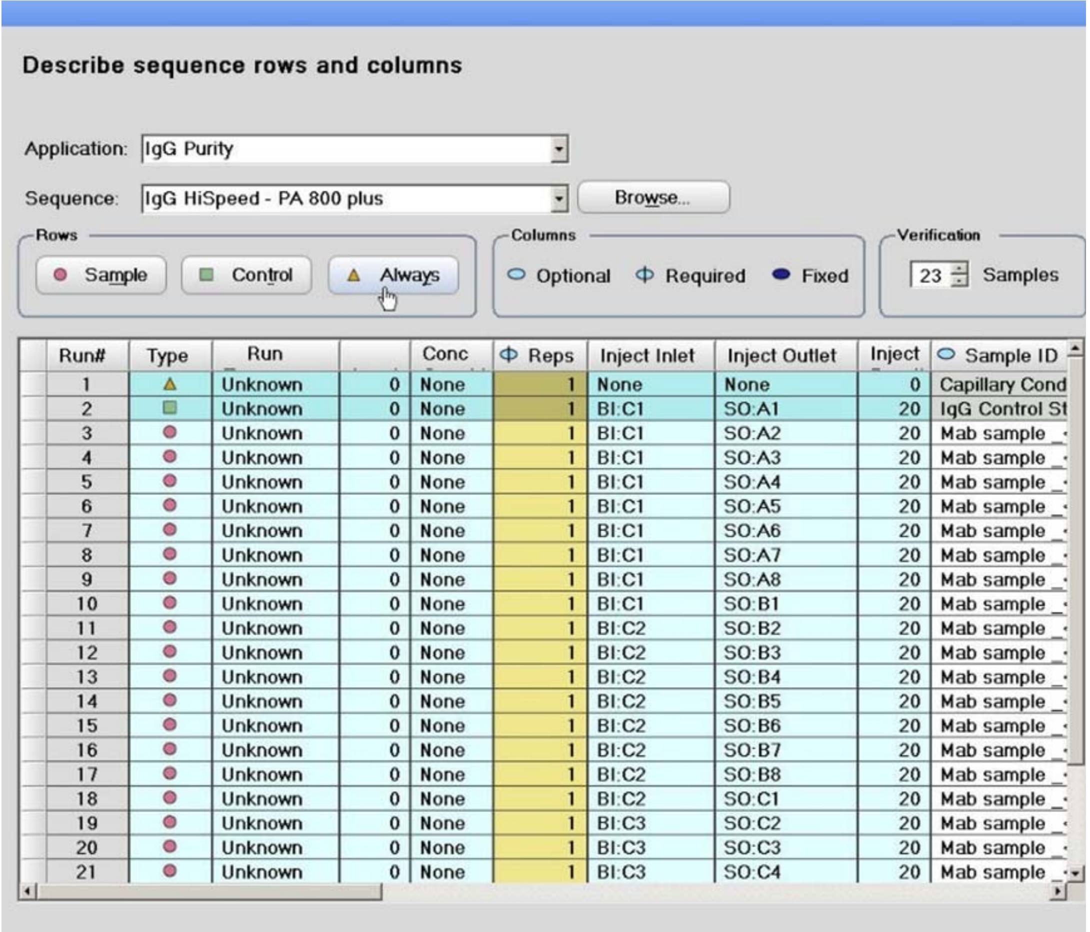
8 (Optional) Edit the **Sample ID** and **Data** fields as desired.

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 as Sample, Control, or Always. Click the row to select it and then click the button in the Rows area.

In Figure 7, Capillary Conditioning and Shutdown runs are set as Always. Run #2 is set as Control. Sample ID is set as Optional. Reps are set as Required.

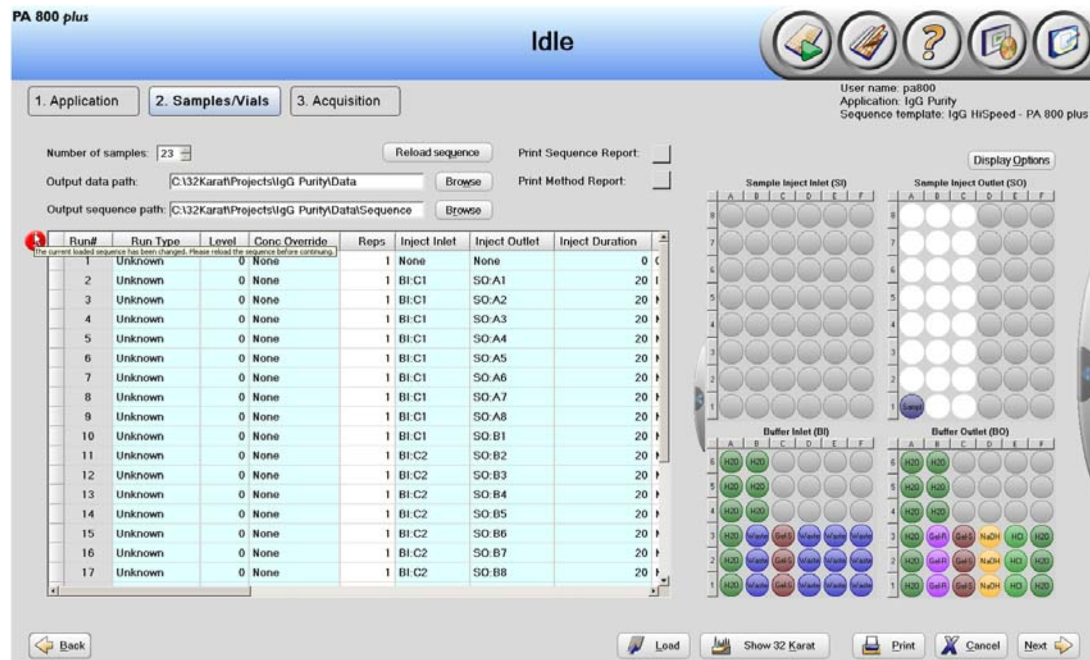
Figure 7 Describe sequence rows and columns Window – Conditioning Method Set to “Always”



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 (Figure 8).

Figure 8 Describe sequence rows and columns Window – Reload Sequence



NOTE In the upper left corner next to Run #1 of the table in Figure 8, a blinking red exclamation mark indicates that the sequence has changed and 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.

Another example of a required action is in the case of a data file name being 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 8 and then close the door.

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

Figure 9 PA 800 Plus Software: Run Window

Capillary Cleaning and Storage

For short-term storage on the instrument (< 10 days), clean and store the capillary following separation:

Perform a shutdown method to clean the capillary. Leave the capillary ends dip in water vials.

For long-term storage (> 10 days), clean and store the capillary following separation:

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 instrument.
4. Store the cartridge in the cartridge box with the inlet and outlet ends in water vials.

5 Keep the cartridge upright in the box.

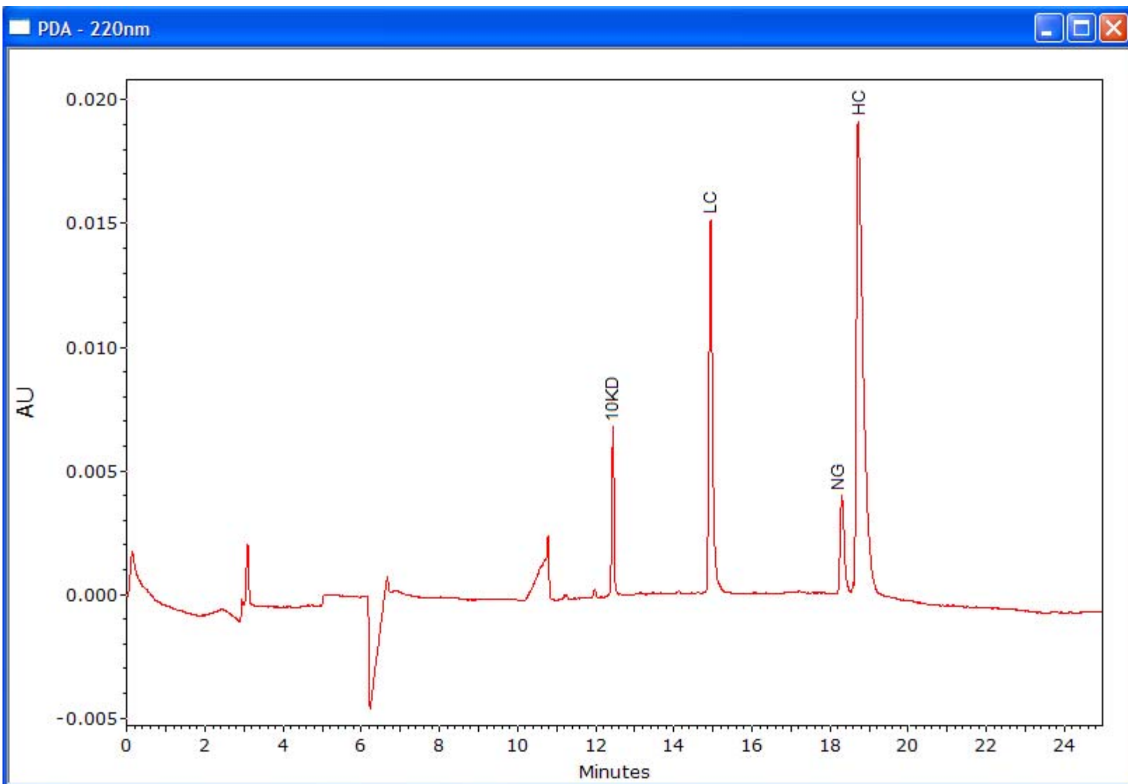
Evaluate the Results

Typical Results for 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 quantification benchmark. Figure 10 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 IgG light chain (LC), heavy chain (HC), 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 10 Separation of IgG Control Standard Using Reduced Conditions

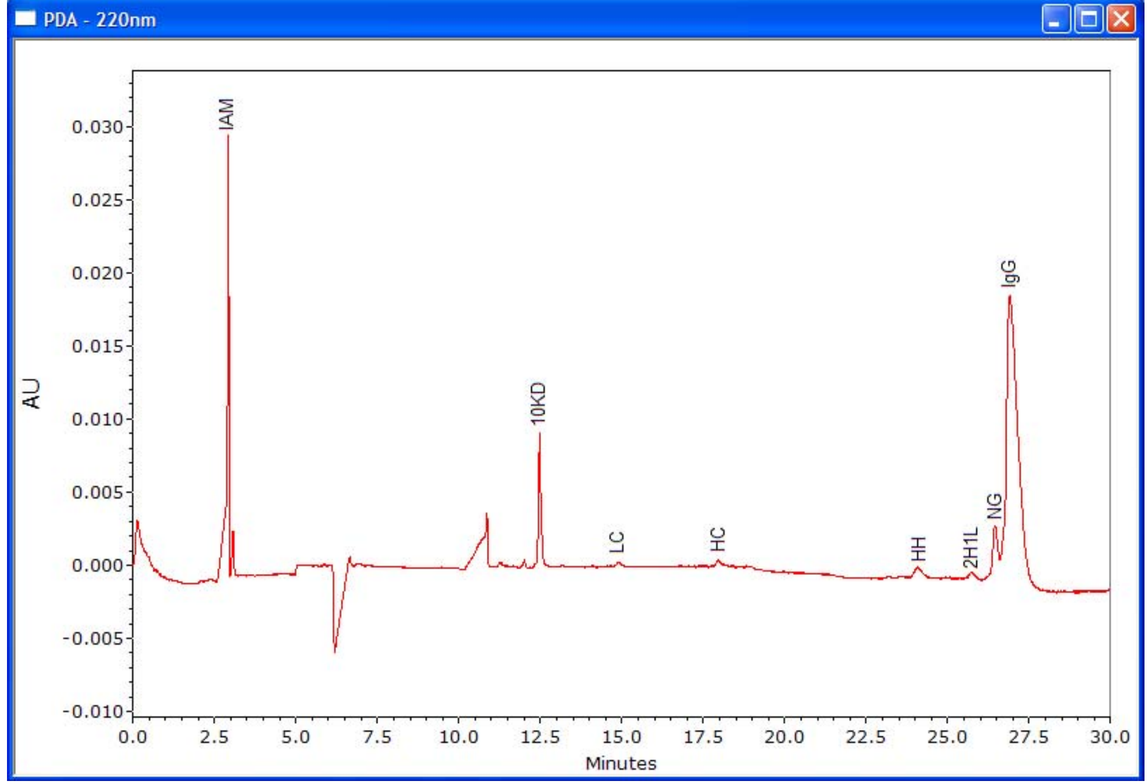


LC	Light Chain	HC	Heavy Chain
NG	Non-Glycosylated Heavy Chain	10 kD	Internal Standard

Typical Results for Non-reduced IgG Control Standard

Under non-reducing conditions, all the impurities, such as light chain (LC), heavy chain (HC), heavy-heavy chain (HH) and 2 heavy 1 light chain (2H1L), resolve from the whole antibody. Refer to Figure 11.

Figure 11 Electropherogram of IgG Control Standard Using Non-Reducing Conditions



Troubleshooting

Table 4 Troubleshooting

Problem	Possible Cause	Corrective Action
Failed system suitability test	Capillary length incorrect or reagents and samples not properly positioned.	Inspect the capillary length, buffer tray, and sample tray first. Make sure 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.
Low or erratic current	Capillary plugged	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 *PA 800 Plus System Maintenance Guide*.
Spikes in electropherogram	Air bubbles in gel buffer	Degas SDS-MW gel buffer under 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 recommended procedure. Use fresh 2-mercaptoethanol for sample reduction. Refer to Prepare the IgG Reduced 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.

Table 4 Troubleshooting

Problem	Possible Cause	Corrective Action
No peaks or low signal	Capillary inlet longer than the inlet electrode	Push the capillary up into the cartridge or cut the capillary inlet to make sure 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. 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.

Hazard classification according to HCS 2012.

Hazardous Substances

Acidic Wash 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.

IgG Control Standard

WARNING! Causes mild skin irritation.

SDS-MW Gel Buffer - proprietary formulation, pH 8, 0.2% SDS

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

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, A51967AE, 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.

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 www.sciex.com *and download the latest version of the guide.*