Application Guide

PA 800 *plus* Pharmaceutical Analysis System

IgG Purity/Heterogeneity Assay

A51967AD
January 2014

Beckman Coulter, Inc.
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BECKMAN COULTER

Application Guide
PA 800 *plus* Pharmaceutical Analysis System
IgG Purity/Heterogeneity Assay
A51967AD (January 2014)

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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

Symbols and Labels

Introduction

The following is a description of symbols and labels used on the Beckman Coulter PA 800 plus Pharmaceutical Analysis System or shown in this manual.

⚠ WARNING

If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the instrument may be impaired.

General Biohazard Symbol

This caution symbol indicates a possible biohazard risk from patient specimen contamination.

A011460L.PNG

Caution, Biohazard Label

This caution symbol indicates a caution to operate only with all covers in position to decrease risk of personal injury or biohazard.

A011459L.EPS

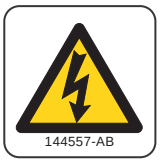
Caution, Moving Parts Label

This caution symbol warns the user of moving parts that can pinch or crush.

A015081L.EPS

High Voltage Electric Shock Risk Symbol

This symbol indicates that there is high voltage and there is a risk of electric shock when the user works in this area.



A016352L.EPS

Class 1 Laser Caution Label

A label reading “Complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Noticed No. 50, dated June 24, 2007” is found near the Name Rating tag. The laser light beam is not visible.

Sharp Object Label

A label reading "CAUTION SHARP OBJECTS" is found on the PA 800 *plus*.

A016351L.EPS

Recycling Label

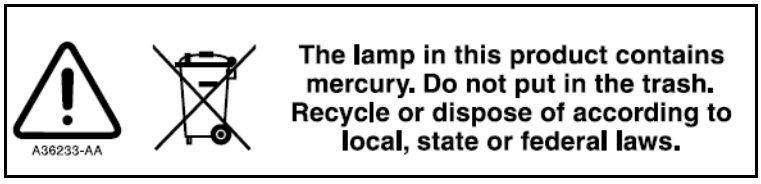
This symbol is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates:

1. The device was put on the European Market after August 13, 2005.
2. The device is not to be disposed of via the municipal waste collection system of any member state of the European Union.

A016608L.PNG

It is very important that customers understand and follow all laws regarding the proper decontamination and safe disposal of electrical equipment. For Beckman Coulter products bearing this label, please contact your dealer or local Beckman Coulter office for details on the take back program that facilitates the proper collection, treatment, recovery, recycling, and safe disposal of this device.

Disposal of Devices Containing Mercury Components



This product contains a mercury-added part. Recycle or dispose of according to local, state, or federal laws. It is very important that you understand and comply with the safe and proper disposal of devices containing mercury components (switch, lamp, battery, relay, or electrode). The mercury component indicator label can vary depending on the type of device.

Restriction of Hazardous Substances (RoHS) Labels

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

China RoHS Caution Label — This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

China RoHS Environmental Label — This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center “e” indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

Alerts for Warning, Caution, Important, and Note

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury. The warning can be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. The caution can be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT notice adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

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CHAPTER 1

IgG Purity/Heterogeneity Assay

Introduction

The PA 800 *plus* IgG Purity/Heterogeneity Assay includes methods to resolve both reduced and non-reduced immunoglobulins by size, and to subsequently quantify the heterogeneity and impurities which may exist in a given 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 (i.e., with 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), while high-speed (HS) methods provide faster separation (in about 15 minutes) with some reduction in resolution. This standard operating procedure (SOP) is specified using the high-resolution methodology. Information on High-Speed Separation is found in CHAPTER 2, *High-Speed Separation Methods*.

Visit www.beckmancoulter.com for the most current versions of the methods.

NOTE This application guide has been validated for use in PA 800 Enhanced and PA 800 *plus* Pharmaceutical Analysis Systems.

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

Intended Use

The IgG Purity Kit is **for laboratory use only**. It is not for use in diagnostic procedures. No clinical decision or patient notification may be made based on results using this laboratory assay.

⚠ CAUTION

Refer to the Material Safety Data Sheets (MSDS) information, available at www.Beckmancoulter.com, regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

Kit Contents

Table 1.1 Contents of this Kit (Reorder PN A10663)

Component	Quantity
Capillary, 50 µm I.D. bare-fused silica	2
SDS-MW Gel Buffer - proprietary formulation, pH 8, 0.2% SDS	140 mL
SDS-MW Sample Buffer - 100 mM Tris-HCl, pH 9.0, 1% SDS	50 mL
IgG Control Standard	1 mL
Internal Standard, 10 kDa protein, 5 mg/mL	0.4 mL
Acidic Wash Solution, 0.1 N HCl	100 mL
Basic Wash Solutio, 0.1 N NaOH	100 mL

Table 1.2 Replacement Reagents

Component	Quantity	Part Number
Capillary, 50 µm I.D. bare-fused silica	pkg of 3	338451
SDS-MW Gel Buffer - proprietary formulation, pH 8, 0.2% SDS	pkg of 4	A30341
IgG Control Standard, 1 mg/mL	pkg of 3	391734
Internal Standard, 10 kDa protein, 5 mg/mL	pkg of 1	A26487

Storing the Assay Components

Reagents

Upon receipt, store the 10 kDa Internal Standard at 2°C to 8°C. The IgG control standard must be aliquoted on 95 µL volumes and stored promptly at -20°C. Capillary, Sample Buffer, SDS-MW Gel Buffer, Acid wash solution, and Basic wash solution should be stored at room temperature.

If precipitate is noted in the SDS-MW Gel Buffer or Sample Buffer, stir it before use at room temperature until the precipitate is fully dissolved. Before starting a CE separation, SDS-MW Gel Buffer and Sample Buffer should be brought to room temperature for at least four hours.

Table 1.3 Materials Required but not provided by Beckman Coulter

Component	Part Number
2-mercaptoethanol	Sigma, PN M7154
Water Bath (37°C to 100°C) or Heat Block	
Centricon YM-30 Centrifugal Filter Unit	Millipore, PN 4208
Iodoacetamide	Sigma PN I-1149
Parafilm	
Sonicator	
Vortexer	
Pipets of various sizes with corresponding pipet tips	
Micro-centrifuge	
Double-deionized (DDI) Water	

Table 1.4 Beckman Coulter Consumables

Component	Part Number
0.5 mL Micro-centrifuge Capped Vials (pack of 500)	344319
1.5 mL Centrifuge Tubes (pack of 500)	357448
Universal Plastic Vials (pack of 100)	A62251
Universal Rubber Vial Caps - blue (pack of 100)[a]	A62250
200 µL Micro Vials (pack of 50)	144709

a. Vial caps are one-time-use only and are not intended for re-use.

Preparing the PA 800 *plus* Instrument

NOTE Before proceeding, you must understand the following procedures as described in the PA 800 *plus* System Maintenance Guide (PN A51964AD):

- Capillary Replacement
- Installation of the PDA detector
- How to calibrate the PDA detector
- How to load and unload trays

Installing the Capillary

1 Install a 50 µm i.d. bare fused-silica capillary into a PA 800 *plus* cartridge set for a total capillary length of 30.2 cm.

The IgG Purity/Heterogeneity assay is optimized using a 30.2 cm capillary with a 20.0 cm effective length from the sample introduction inlet to the center of the detector window.

2 Use the 100 x 200 µm capillary aperture for this installation.

To have good reproducibility from capillary to capillary and accurate mobility assignments, it is important to adhere to the capillary pre-measurement procedure.

NOTE The cut ends of capillaries should be inspected carefully under magnification. The cut must be clean (not jagged) and perpendicular to the capillary length (not angled). Poor cuts result in poor resolution and poor sample loading.

3 Turn off the PA 800 *plus* instrument and install the PDA detection module.

4 Turn on the instrument and permit the UV lamp to warm up for at least 30 minutes prior to experimentation.

Cleaning the Interface

Clean the electrodes, capillary ends, opening levers, and interface block carefully, following the cleaning procedure as described in the PA 800 plus "System Maintenance" manual, either once a day or after the finish of the sequence. The SDS 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 employed. Gel accumulation may cause various modes of system failure including broken capillaries, bent electrodes, vial jams, and missed injections.

Inserting the Cartridge/Calibrating the PDA

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.

Sample Preparation

Preparing the IgG Control Standard

1. Take one vial of the 95 µL aliquots of the IgG control standard and set it at room temperature until it is completely thawed.
2. Add two µL of 10 kDa Internal Standard to the IgG tube.
3. Inside a fume hood, add five µL of 2-mercaptoethanol to the IgG tube.
4. Cap the tube and mix thoroughly.
5. Centrifuge at 300 g for 1 minute.
6. Seal the vial cap with Parafilm and heat the mixture at 70°C for 10 minutes.
7. Place the vial in a room-temperature water bath to cool for at least 3 minutes.
8. Transfer 100 µL of the prepared sample into a micro vial, place the micro vial into a universal vial, and cap the universal vial.

IgG Sample Preparation

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 Centricon YM- 30 centrifuge filter unit. Follow the instructions provided in *Buffer Exchanging the IgG Sample*.

Reduced IgG Sample Preparation

1. Pipette 100 µg of IgG sample in a volume less than 45 µL into a 0.5 mL micro-centrifuge vial.
2. Add 50 to 95 µL of sample buffer to give a final volume of 95 µL.
3. Add two µL 10 kDa Internal Standard to the IgG sample tube.
4. Inside a fume hood, add five µL of 2-mercaptoethanol to the IgG sample tube.
5. Cap the vial tightly and then mix thoroughly.
6. Centrifuge at 300 g for 1 minute.
7. Seal the tube with Parafilm and heat the mixture at 70°C for 10 minutes.
8. Place the vial in a room-temperature water bath to cool for at least 3 minutes before transferring it into the sample vial.
9. Transfer 100 µL of the prepared sample into a micro vial. Place the micro vial inside a universal vial and cap the universal vial.

Buffer Exchanging 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 in your IgG sample is too high, low signal and peak tailing will be observed. Buffer exchange the sample with a Centricon tube using the following procedure:

1. Add one mL of IgG sample to Centricon YM-30.
2. Centrifuge at 4,000 g for 15 minutes.
3. Add 2 mL of SDS-MW Sample Buffer, then centrifuge at 4,000 g for 25 min.

4 Insert the centricon upside-down to drain the suspended IgG solution (in the filter membrane) into a new vial and centrifuge for three min. at 1,000 g.

5 Transfer the collected IgG to an appropriate tube.

6 Add SDS-MW Sample Buffer to give a final volume of one mL.

Non-reduced IgG Sample Preparation

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 your IgG sample using the following procedure:

Preparing Alkylation Reagent

A 250 mM iodoacetamide (IAM) solution is used as the alkylation reagent. The solution is stable for approximately 24 hours at room temperature.

1 Weigh 46 mg of high purity IAM.

2 Transfer the IAM into 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 and mix thoroughly until dissolved.

5 Make this mix fresh daily and do not expose it to light.

Preparing IgG Non-reduced Sample

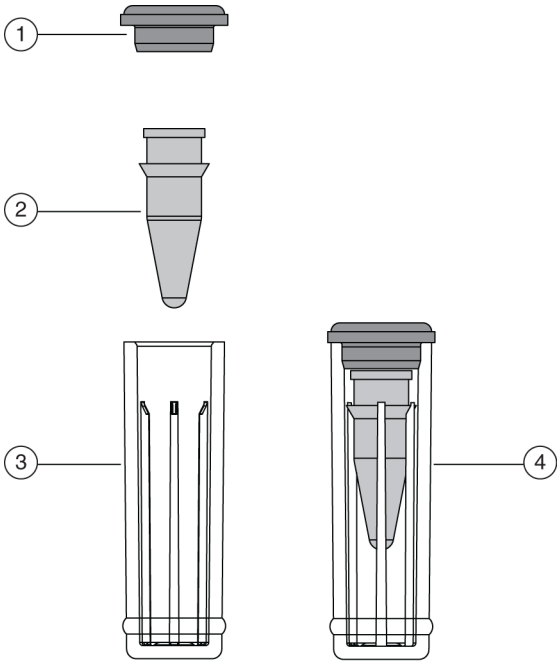
1. Pipette 100 µg of IgG sample into a 0.5 mL micro-centrifuge tube.
2. Add from 50 to 95 µL of sample buffer to give a final volume of 95 µL.
3. Add 2 µL of Internal Standard into the tube.
4. Add 5 µL of the 250 mM IAM solution into the sample tube.
5. Cap the vial tightly and mix thoroughly.
6. Centrifuge at 300 g for 1 minute.
7. Seal the tube with Parafilm and heat the mixture in a water bath at 70°C for 10 minutes.
8. Place the tube in a room temperature water bath to cool for at least 3 minutes.
9. Transfer 100 µL of the prepared sample into a 200 µL micro vial and spin down the contents to remove any air bubbles. Place the micro vial inside a universal vial and cap the universal vial.

Sample Vial Setup

Before placing the 200 microliter sample vials into the universal vials, ensure that no bubbles are at the bottom of the sample vials. If bubbles exist, centrifuge the sample vials for 2 minutes at 1,000 g and repeat if necessary. Place a cap on the universal vial and ensure a good seal. See Figure 1.1.

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

Figure 1.1 Micro Vial Inside Universal Vial



901927L.AI

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

Buffer Vial Preparation and Loading

One 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, but sample number one must always be the IgG control standard.

Fill the appropriate number of reagent vials with the SDS-MW Gel Buffer, 0.1 N NaOH solution, 0.1 N HCl solution, and DDI water according to the information under Tray Configuration.

The number of reagent vials is dependent upon 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 for every eight cycles run. The buffer tray templates illustrated in Table 1.5, Inlet Buffer Tray Schematic, and Table 1.6, Outlet Buffer Tray Schematic are set up for use with the high-resolution methods, which introduce the sample from the left-side tray.

Preparing the Reagent Vials

1. Fill the gel-rinse (Gel-R) vials with 1.2 mL of SDS-MW Gel Buffer. Fill the gel separation (Gel-S) vials with 1.1 mL of SDS-MW Gel Buffer.

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

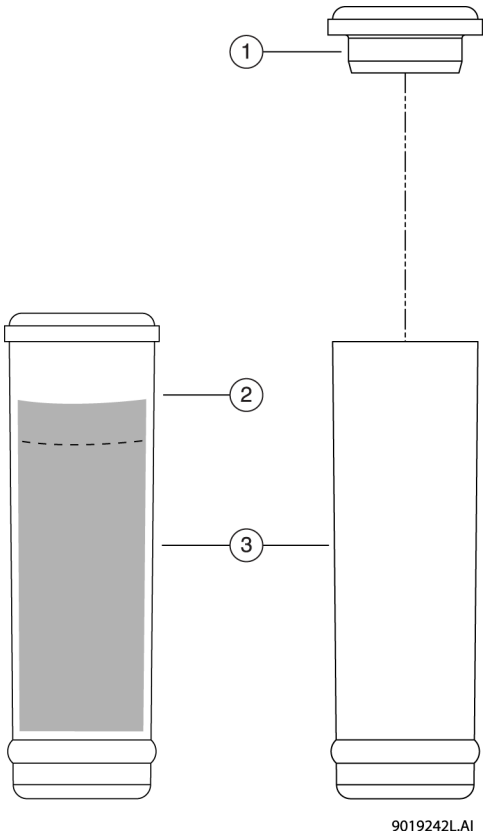
3. Fill the NaOH and HCl vials to 1.5 mL.

4. Fill the waste vials with 0.8 mL of DDI water.

⚠ WARNING

Pressure system damage can occur when the waste vial volume exceeds 1.8 mL.

Figure 1.2 Universal Vials and Caps



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

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

5 Cap the universal vials.

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

Tray Configuration

1 Load the test reagents into the system inlet (left) and outlet (right) 6x6 buffer trays using the configuration illustrated in Table 1.5, Inlet Buffer Tray Schematic and Table 1.6, Outlet Buffer Tray Schematic.

2 Load the trays into the PA 800 *plus* system.

Inlet Buffer Tray

Table 1.5 Inlet Buffer Tray Schematic

H_2O (Cycle 17-24)	H_2O (Cycle17-24)				
H_2O (Cycle 9-16)	H_2O (Cycle 9-16)				
H_2O (Cycle 1-8)	H_2O (Cycle 1-8)				
H_2O (Cycle 17-24)	Gel-R (Cycle 17-24)	Gel-S (Cycle 17-24)	NaOH (Cycle 17-24)	HCl (Cycle 17-24)	H_2O (Cycle 17-24)
H_2O (Cycle 9-16)	Gel-R (Cycle 9-16)	Gel-S (Cycle 9-16)	NaOH (Cycle 9-16)	HCl (Cycle 9-16)	H_2O (Cycle 9-16)
H_2O (Cycle 1-8)	Gel-R (Cycle 1-8)	Gel-S (Cycle 1-8)	NaOH (Cycle 1-8)	HCl (Cycle 1-8)	H_2O (Cycle 1-8)
A	B	C	D	E	F

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 fill capillary prior each cycle, 1.2 mL.

C1 to C3 – SDS-MW Gel Buffer for separation, 1.1 mL.

D1 to D3 – 0.1N NaOH, use to precondition capillary, 1.5 mL.

E1 to E3 – 0.1N HCl, use to precondition capillary, 1.5 mL.

F1 to F3 – DDI H_2O, use to precondition capillary, 1.5 mL.

Outlet Buffer Tray

Table 1.6 Outlet Buffer Tray Schematic

H_2O (Cycle 17-24)	H_2O (Cycle 17-24)				
H_2O (Cycle 9-16)	H_2O (Cycle 9-16)				
H_2O (Cycle 1-8)	H_2O (Cycle 1-8)				
H_2O (Cycle 17-24)	Waste (Cycle 17-24)	Gel-S (Cycle 17-24)	Waste (Cycle 17-24)	Waste (Cycle 17-24)	Waste (Cycle 17-24)
H_2O (Cycle 9-16)	Waste (Cycle 9-16)	Gel-S (Cycle 9-16)	Waste (Cycle 9-16)	Waste (Cycle 9-16)	Waste (Cycle 9-16)
H_2O (Cycle 1-8)	Waste (Cycle 1-8)	Gel-S (Cycle 1-8)	Waste (Cycle 1-8)	Waste (Cycle 1-8)	Waste (Cycle 1-8)
A	B	C	D	E	F

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, 0.8 mL of DDI water.

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

D1 to D3: Waste vial for 0.1N NaOH rinse, 0.8 ml of DDI water.

E1 to E3: Waste vial for 0.1N HCl rinse, 0.8 ml of DDI water.

F1 to F3: Waste vial for DDI H_2O rinse, 0.8 ml of DDI water.

Running the Assay

Launching the IgG Purity Instrument

1 Launch the 32 Karat software. The Enterprise screen displays the available instruments.

2 Double-click on the **IgG Purity** instrument to launch the IgG purity/heterogeneity assay.

3 Select **IgG Purity** as the project on the log in screen.

NOTE Type **PA 800** as the user name and type **Plus** as the password.

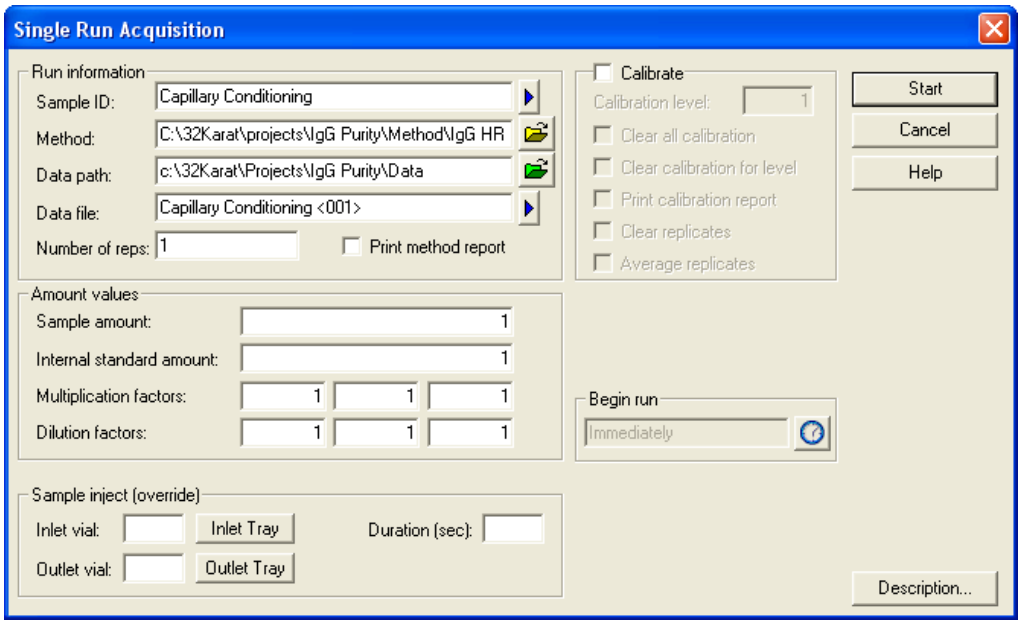
NOTE User Name and Password defaults have been established for installation and training purposes.

Conditioning a New Capillary

A new capillary needs to be conditioned once before use.

1 To initiate the conditioning method, click **Control > Single Run** from the 32 Karat Menu bar. The Single Run Acquisition dialog box opens.

Figure 1.3 Single Run Acquisition Dialog Box



2 Select the **IgG HR Conditioning - PA 800 *plus*** and type in the data file name of your choice.

3 Click on the **Arrow** button and select the **Increment** option.

4 Type 1 as the **Number of Reps**.

Since no sample is injected, a vial injection position does not need to be specified.

5 Select **Start** to begin the conditioning process.

Running IgG Controls and Test Samples

The sequence table is designed to automate the process of running controls and samples.

For your convenience, two sequence templates have been pre-programmed to run up to 24 samples using the high-resolution methods. The sequence table has been designed this way to consider the automatic replacement of the assay reagents during the course of the experiment.

If you mix different methods within the same sequence, you must consider the impact on the reagents in the buffer trays and ensure they are replenished as needed.

Once the buffer trays and samples have been loaded into the system, you are ready to begin the analysis of the test compounds.

NOTE Check the Beckman Coulter web site for the most current versions of the pre-programmed sequences and methods for this assay. Visit www.beckmancoulter.com and select the appropriate files.

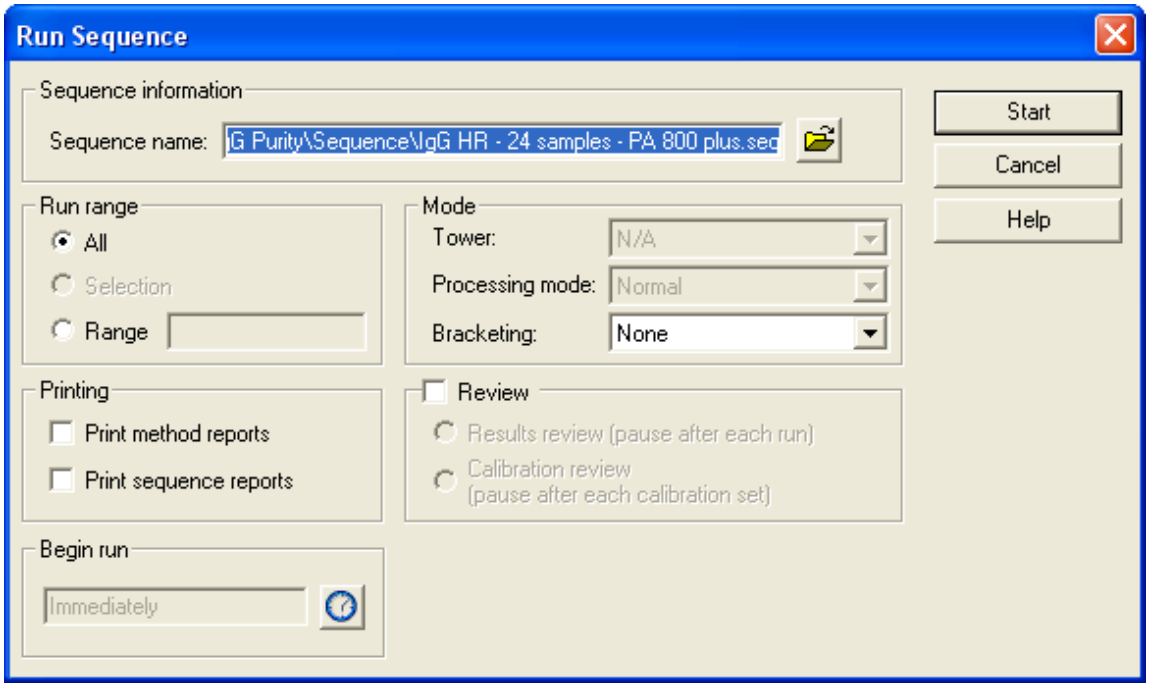
1 On the Task bar, click **Control**, then click **Sequence Run**. The Run Sequence dialog box opens. See Figure 1.4.

2 Click on the **Folder** button to browse to the proper IgG sequence.

3 Set the **Run Range** to **All**.

4 Click **Start**.

Figure 1.4 Starting a Sequence



Alternatively, you can edit the sequence table to include specific sample names for each of your test compounds. You can also specify whether to run a shutdown method after the end of the sequence. An example of sequence template pre-programming to run suitability — 24 runs and a shutdown method — is shown in Figure 1.5.

The shutdown method should be run at the end of the experimentation. This method cleans the capillary and turns off the UV lamp.

Figure 1.5 Sequence Template Pre-programming to Run Suitability

Sequence: IgG HR - 24 samples - PA 800 plus.seq

Run #	Status	Run Type	Level	Conc Override	Custom	Reps	Sample Inject Inlet	Sample Inject Outlet	Sample Inject Duration	Sample ID	Method	Filename
1		Unknown	0	n/a	Unconfigured	1				Capillary Conditioning	hing - PA 800 plus.met	y Conditioning<D>.dat
2		SSB SSE SMB SME	0	n/a	Unconfigured	1	SI:A1	BO:C1	20.0	IgG Control Standard	aration - PA 800 plus.met	<D>.dat
3		Summary Begin	0	n/a	Unconfigured	1	SI:A2	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
4		Summary Run	0	n/a	Unconfigured	1	SI:A3	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
5		Summary Run	0	n/a	Unconfigured	1	SI:A4	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
6		Summary Run	0	n/a	Unconfigured	1	SI:A5	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
7		Summary Run	0	n/a	Unconfigured	1	SI:A6	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
8		Summary Run	0	n/a	Unconfigured	1	SI:A7	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
9		Summary Run	0	n/a	Unconfigured	1	SI:A8	BO:C1	20.0		aration - PA 800 plus.met	<D>.dat
10		Summary Run	0	n/a	Unconfigured	1	SI:B1	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
11		Summary Run	0	n/a	Unconfigured	1	SI:B2	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
12		Summary Run	0	n/a	Unconfigured	1	SI:B3	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
13		Summary Run	0	n/a	Unconfigured	1	SI:B4	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
14		Summary Run	0	n/a	Unconfigured	1	SI:B5	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
15		Summary Run	0	n/a	Unconfigured	1	SI:B6	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
16		Summary Run	0	n/a	Unconfigured	1	SI:B7	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
17		Summary Run	0	n/a	Unconfigured	1	SI:B8	BO:C2	20.0		aration - PA 800 plus.met	<D>.dat
18		Summary Run	0	n/a	Unconfigured	1	SI:C1	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
19		Summary Run	0	n/a	Unconfigured	1	SI:C2	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
20		Summary Run	0	n/a	Unconfigured	1	SI:C3	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
21		Summary Run	0	n/a	Unconfigured	1	SI:C4	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
22		Summary Run	0	n/a	Unconfigured	1	SI:C5	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
23		Summary Run	0	n/a	Unconfigured	1	SI:C6	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
24		Summary Run	0	n/a	Unconfigured	1	SI:C7	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
25		Summary End	0	n/a	Unconfigured	1	SI:C8	BO:C3	20.0		aration - PA 800 plus.met	<D>.dat
26		Shutdown	0	n/a	Unconfigured	1					utdown - PA 800 plus.met	

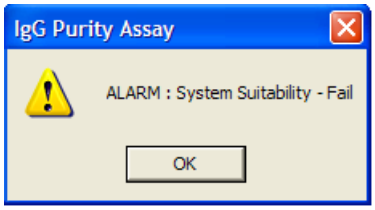
Checking the Separation Results

System Suitability Results

The first run in any given sequence should be the system suitability test using the control standard. After running this suitability test, the software automatically analyzes the data and summarizes it in a suitability test report. If the run does not pass the criteria for the suitability test, an alarm sounds and displays. See Figure 1.6.

Alternatively, you can bracket your test compounds with an IgG control standard on either side, in which case, suitability would be determined only after all the samples have been processed.

Figure 1.6 Visual Alarm with Suitability Failure



Failure of System Suitability serves to abort the sequence with this template. The suitability parameters designated are relatively straightforward. The system must identify the 10 kDa internal standard, the light chain, heavy chain, and non-glycosylated heavy chain. Furthermore, the resolution between the non-glycosylated heavy chain and the heavy chain must be greater than one. If the IgG control fails suitability, then your assay is not suitable to run. Consult the Troubleshooting Guide in this chapter.

If the suitability test passes, the system automatically starts running your samples according to the programmed sequence. The system also generates a suitability report, which can be printed automatically or manually.

System Suitability Report

To specify the viewing or printing of this report, select reports, and select **View** or **Print Custom Sequence reports.** Next select the **Suitability Report.** See Figure 1.7.

Figure 1.7 System Suitability Report

System Suitability Report Page 1 of 1

Sequence : C:\32Karat\projects\IgG Purity\Sequence\IgG HRR - PA 800 plus.seq
User : PROTEOMELAB
Printed : 2/10/2009 5:04:54 PM

System is Suitable

PDA - 220nm	Compound	Parameter	Min	Max	%RSD
	10kD	mobil		0	
	LC	mobil		0	1
	NG	mobil		0	
	HC	resusp	1	5	
		mobil		0	1

Sample ID	Compound	Parameter	Average	Low	High	%RSD	Status
	10kD	mobil	-0.00005522	-0.00005522	-0.00005522	0.000	
U2-IgG-1		-0.00005522					Passed
	LC	mobil	-0.00004441	-0.00004441	-0.00004441	0.000	
U2-IgG-1		-0.00004441					Passed
	NG	mobil	-0.00003540	-0.00003540	-0.00003540	0.000	
U2-IgG-1		-0.00003540					Passed
	HC	resusp	1.42116	1.42116	1.42116	0.000	
U2-IgG-1		1.42116					Passed
		mobil	-0.00003449	-0.00003449	-0.00003449	0.000	
U2-IgG-1		-0.00003449					Passed

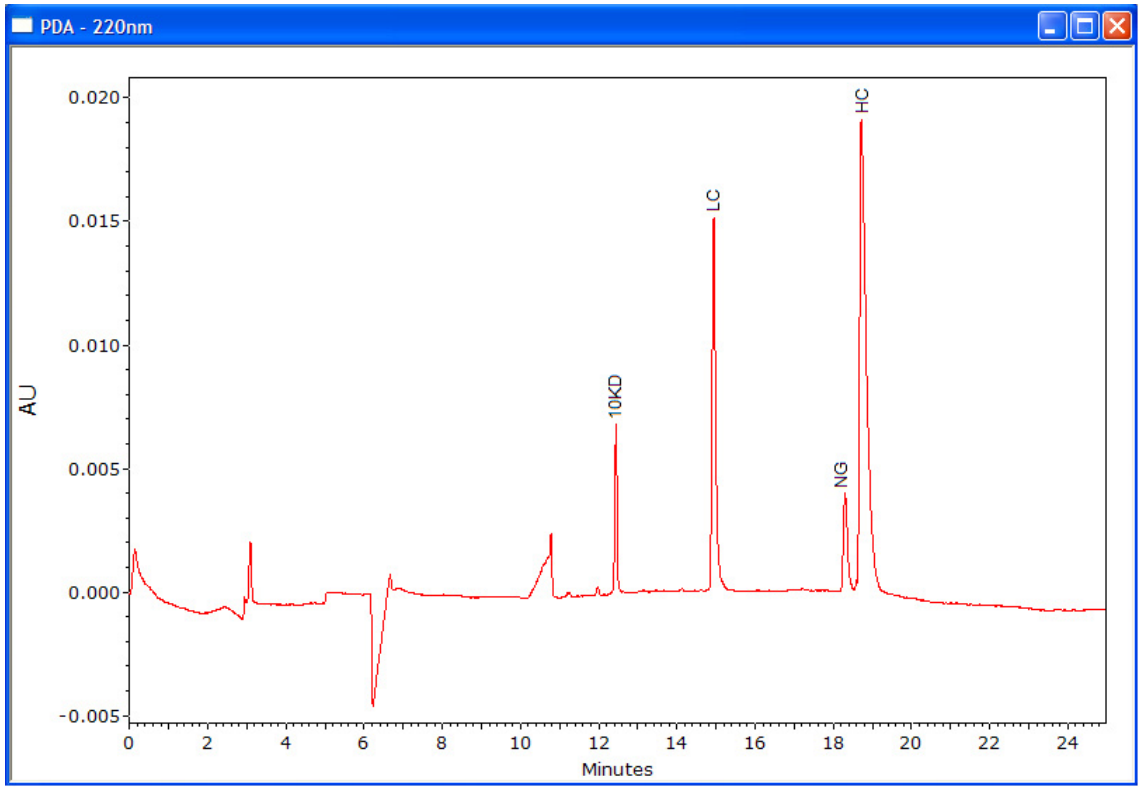
Typical Separation Results

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 1.8 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 (10kD). The glycosylated heavy chain should be baseline resolved from the non-glycosylated heavy chain (resolution >1). The quantification benchmark of the IgG control is identified on the Certificate of Analysis, which is included with the standard, and is expressed as a percentage of the total heavy chain present.

Figure 1.8 Separation of IgG Control Standard Using Reduced Conditions

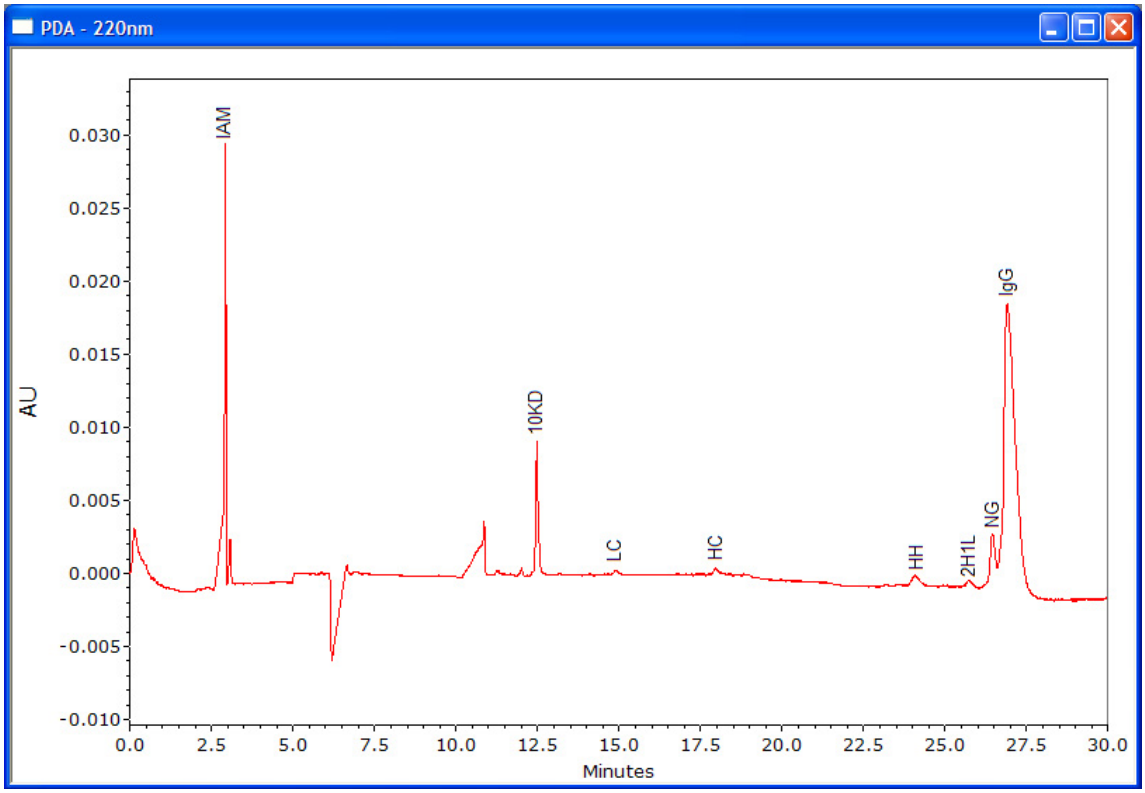


LC	Light Chain	HC	Heavy Chain
NG	Non-Glycosylated Heavy Chain	10 kD	Internal Standard

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. A typical analysis of the IgG control under non-reducing conditions is illustrated in Figure 1.9.

Figure 1.9 Electropherogram of IgG Control Standard Using Non-Reducing Conditions



Troubleshooting Guide

Failed System Suitability Test

Check the capillary length, buffer tray, and sample tray first. Make sure all the reagents and samples are in the right place as described in this Standard Operating Procedure (SOP.) Evaluate the integration and peak identification windows. Next follow the instructions below for additional troubleshooting.

Low or Unsteady Current

Check the current trace. The current should be close to -25 microamperes. If low or unsteady current displays, you see slow migration and poor resolution. As a result, the system suitability test fails.

Possible Cause	Corrective Action
Capillary Plugged	1) Rinse the capillary with DDI water for 10 minutes at 100 psi and then perform the capillary conditioning method. 2) If an unsteady current is still observed, install a new capillary.
Air bubble in the gel	Degas the SDS gel under 5 to 15 Hg vacuum for 5 minutes.
Contamination of the electrode	Clean the electrodes. See the PA 800 *plus* System Maintenance Guide.

Broad Peak and Poor Resolution

Check the raw data for the IgG separation. If broad peaks and poor resolution are observed, follow the instructions below for troubleshooting.

Possible Cause	Corrective Action
Dust or gel build up on capillary end	Clean the capillary end by following the instructions below under *Cleaning the Interface*
Poor capillary end cut	Check the capillary end under magnification. If the capillary end cut is not clean and straight, install a new capillary.
Improper reduction of sample	Reduce sample using recommended procedure. Use fresh mercaptoethanol for sample reduction.
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.

No Peaks or Very Low Signal

If no peaks are observed on the electropherogram, follow the troubleshooting steps below.

Possible Cause	Corrective Action
Capillary inlet is longer than the inlet electrode	Re-adjust by pushing the capillary up or cut the capillary inlet to make sure it is the same length as the electrode.
Dirty or plugged capillary cut	Clean the capillary tip by following the instructions under Cleaning the Interface.
Insufficient quantity of sample	Increase the sample volume in the micro vial to 100 µL.
High salt in IgG sample	Buffer exchange your sample using the procedure under Buffer Exchanging the IgG Sample.
Slow sample migration	Increase the separation time in the method and repeat analysis.

Capillary Cleaning and Storage

Once the capillary has been used for separation, it should be cleaned and stored as follows:

1. For short term storage on the instrument (< 10 days), perform a shutdown method to clean the capillary and fill it with SDS-MW Gel Buffer. The capillary ends dip into water vials.
2. For long term storage, rinse the capillary with DDI water for 10 minutes at 100 psi.
3. Remove the capillary from the instrument.
4. Store the capillary in the cartridge box with the inlet and outlet ends in water vials.
5. Keep the capillary cartridge in the cartridge box in an upright position.

CHAPTER 2

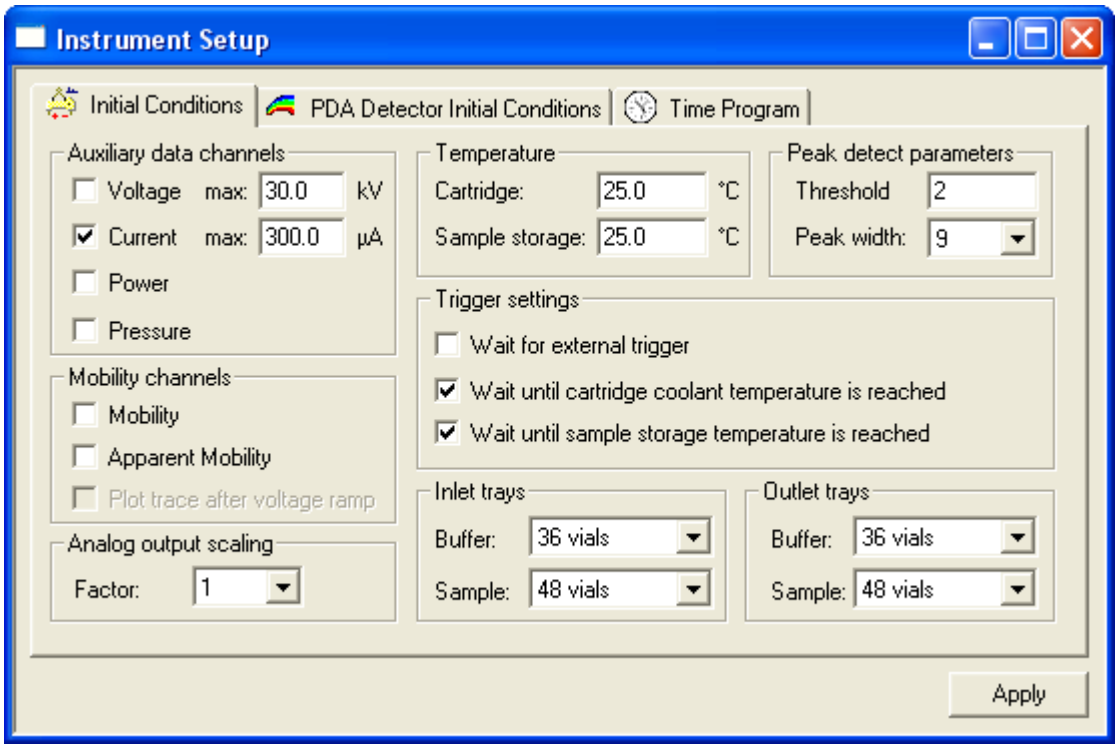
Method Information

Instrument and Detector Initial Conditions

The instrument and detector initial conditions are the same for all IgG methods.

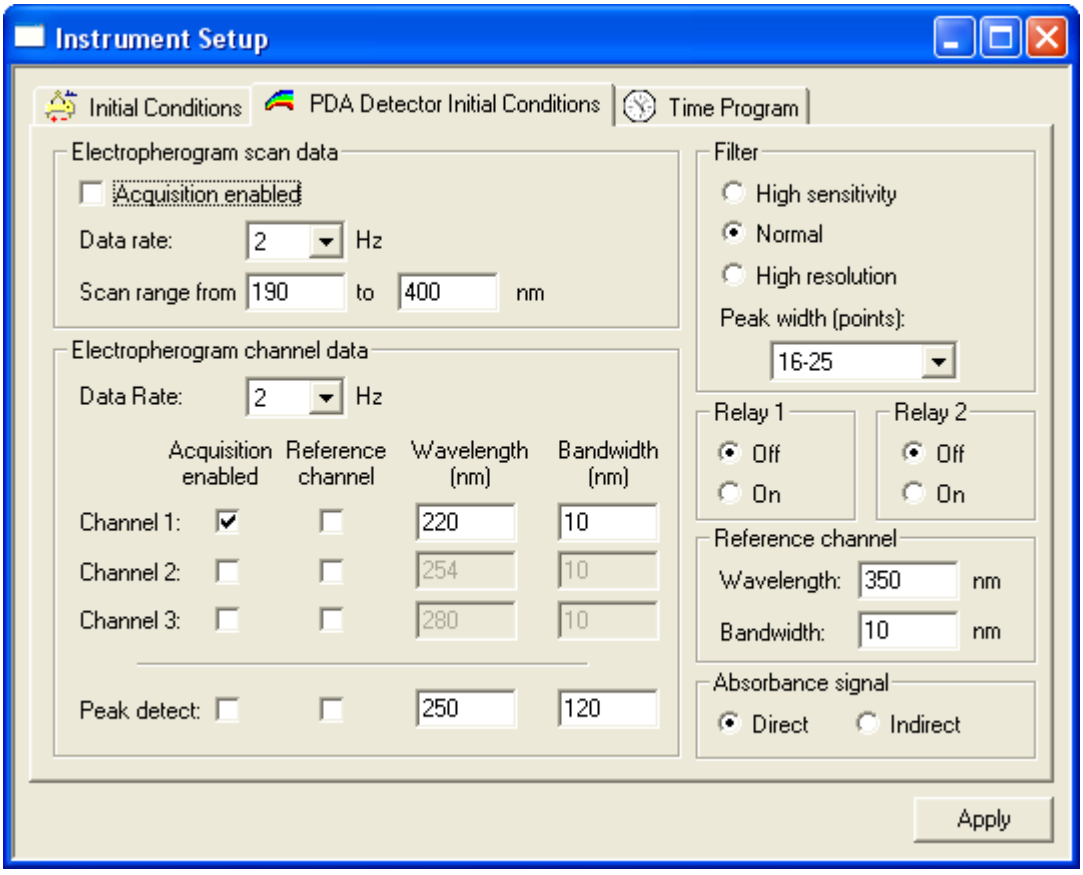
Instrument Initial Conditions (All Methods)

Figure 2.1 Instrument Setup - Instrument Initial Conditions



Detector Initial Conditions (All Methods)

Figure 2.2 Instrument Setup - PDA Detector

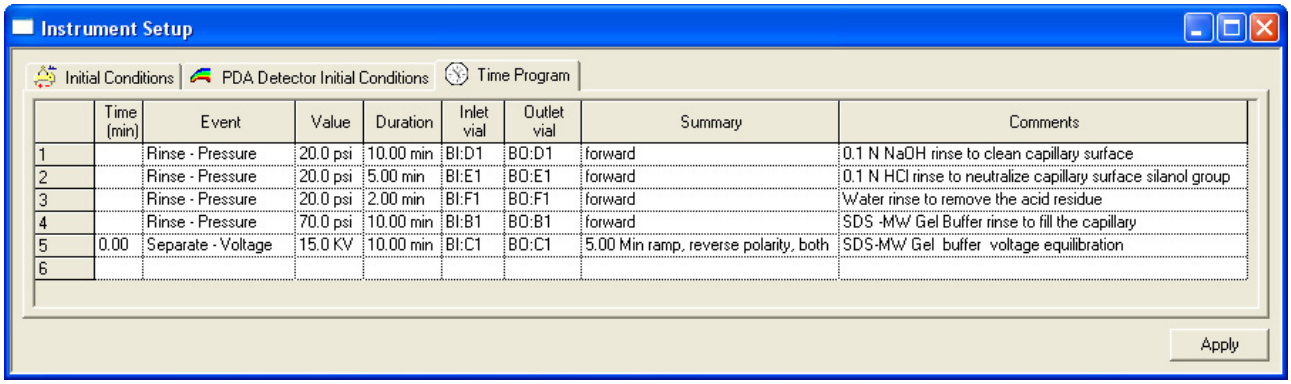


Capillary Precondition Methods

A new capillary — or a used capillary that has been stored for a long period of time — needs to be conditioned using the Capillary Conditioning Method before starting CE separation.

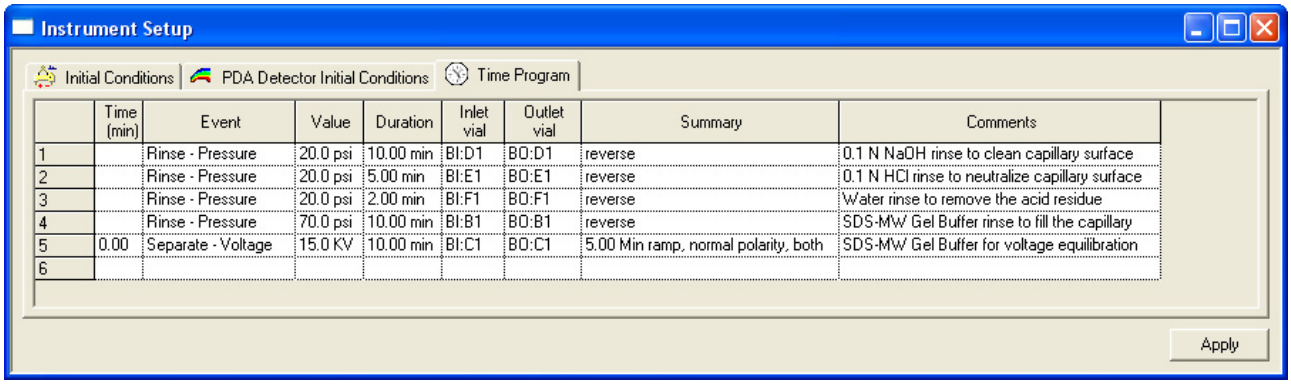
NOTE The high-resolution (HR) method must perform pressure rinses in the forward direction and voltage separation with reverse polarity. In contrast, the high-speed (HS) method must use reverse direction in the pressure rinses and normal polarity in the voltage separation.

Figure 2.3 Time Program - High-Resolution Capillary Preconditioning (IgG HR Conditioning - PA 800 *plus*.met)



	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 2.4 Time Program - High-Speed Capillary Conditioning (IgG HS Conditioning - PA 800 *plus*.met)



	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								

Separation Methods

Two CE methods were optimized for the IgG Purity/Heterogeneity Assay. The High-Resolution (HR) Method employs the capillary cartridge in the left to right orientation (i.e., with an inlet to detection window distance of 20.2 cm). The High-Speed (HS) method, however, employs the cartridge in the opposite orientation, with an inlet to detection window distance of 10 cm.

The High-Resolution method provides high-resolution for protein separation (in about 30 minutes), while the High-Speed method provides faster separation (in about 15 minutes) with slightly lower resolution.

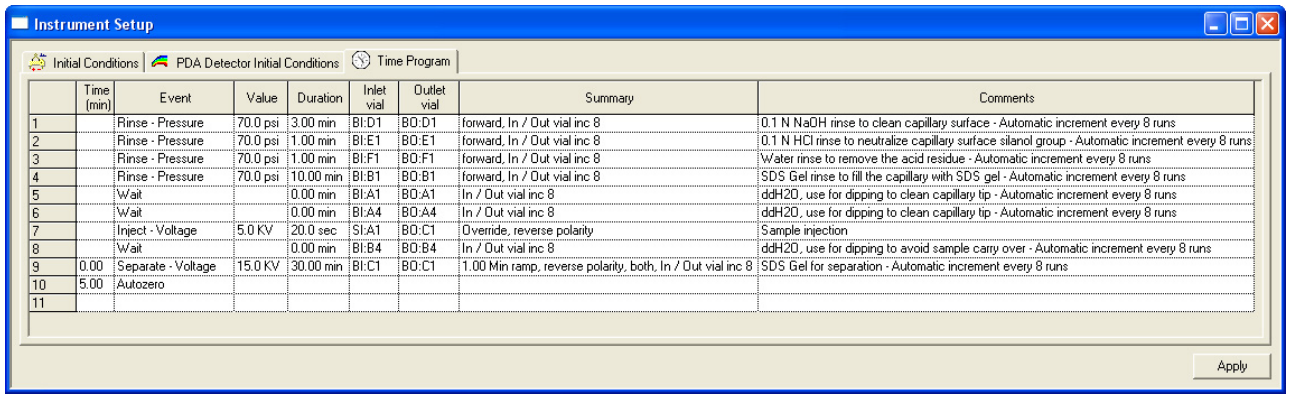
High-Resolution Method

The High-Resolution method employs the capillary cartridge in the left to right orientation (i.e., with an inlet to detection window distance of 20.2 cm). A sequence template is set up for an automatic run sequence of 24 samples. This SDS-CGE methodology is based on run cycles of eight. For each cycle, the capillary is first preconditioned with 0.1 N NaOH, 0.1 N HCl, DDI water and SDS-MW Gel Buffer. Samples are electrokinetically introduced by applying voltage at –5 kV for 20 seconds.

Electrophoresis is performed at constant voltage, with an applied field strength of –497 volts/cm with capillary temperature maintained at 25°C using recirculating liquid coolant. The current

generated is approximately 27 µAmps. The system automatically replenishes all reagents through an increment of the buffer array tray after every eight cycles.

Figure 2.5 High-Resolution Separation Method - (IgG HR Separation - PA 800 *plus*.met)



	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								

High-Speed Separation Methods

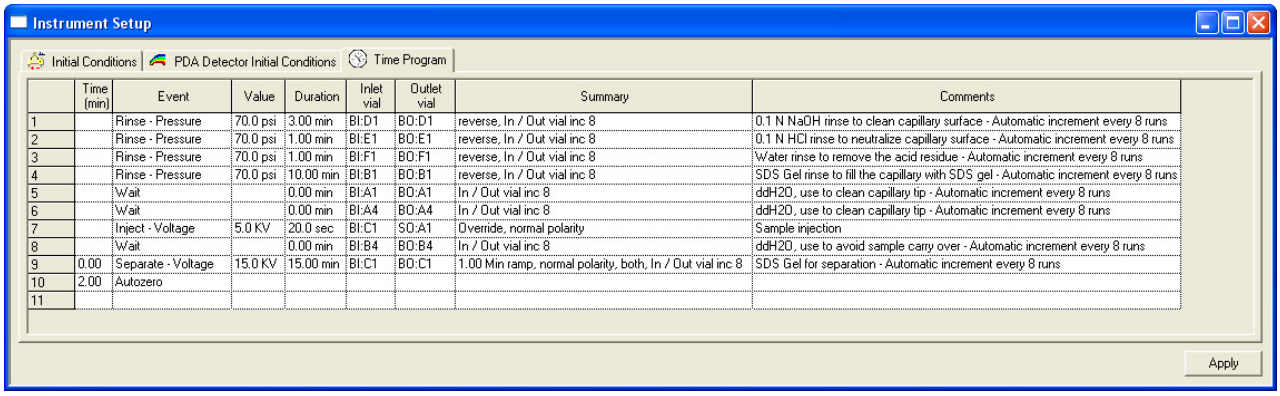
These High-Speed methods employs the cartridge in the opposite orientation, with an inlet to detection window distance of 10 cm. By using these high-speed methods, a faster separation will be obtained (in about 15 minutes) with only slightly lower resolution. This SDS-CGE methodology is based on run cycles of eight.

When these methods are in use, the buffer tray on the right should be used as the inlet tray, and the buffer tray on the left should be used as the outlet tray. The polarity for injection and separation should be **normal**.

For each cycle, the capillary is first preconditioned with 0.1 N NaOH, 0.1 N HCl, DDI water and SDS-MW Gel Buffer. Samples are electrokinetically introduced by applying voltage at –5 kV for 20 seconds. Electrophoresis is performed at constant voltage, with an applied field strength of 497 volts/cm with a capillary temperature maintained at 25°C using recirculating liquid coolant. The current generated is approximately 27 µAmps.

The system automatically replenishes all reagents through an increment of the buffer array tray after every eight cycles.

Figure 2.6 High-Speed Separation Method - (IgG HS Separation - PA 800 *plus*.met)



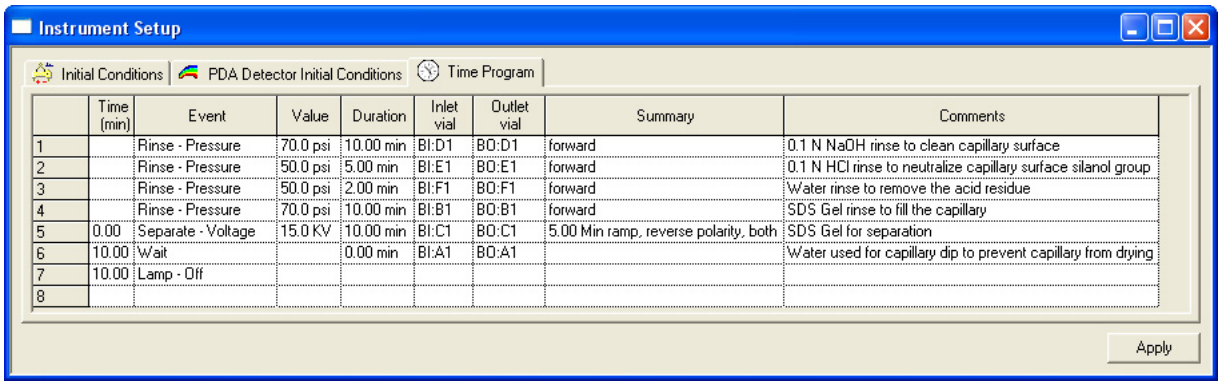
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	reverse, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	reverse, In / Out vial 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:F1	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:B1	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:A1	In / Out vial inc 8	ddH2O, use 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 to clean capillary tip - Automatic increment every 8 runs
7		Inject - Voltage	5.0 KV	20.0 sec	BI:C1	SO:A1	Override, normal polarity	Sample injection
8		Wait		0.00 min	BI:B4	BO:B4	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:C1	1.00 Min ramp, normal polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
10	2.00	Autozero						
11								

Shutdown Methods

A shutdown method should be performed after the sequence is complete. These methods will clean the capillary surface, fill the capillary with fresh SDS-MW Gel Buffer, and turn off the lamp.

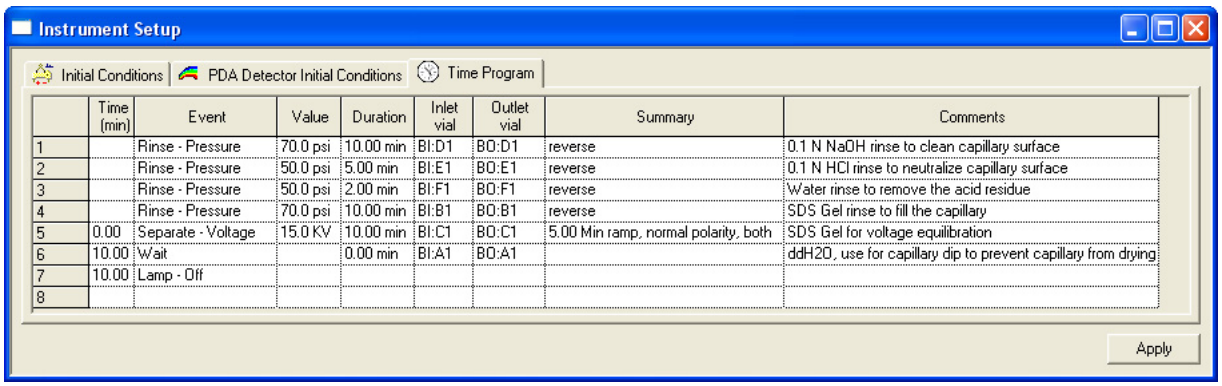
NOTE The high-resolution method must perform pressure rinses in the forward direction and voltage separation with reverse polarity. In contrast, the high-speed method must use reverse direction in the pressure rinses and normal polarity in the voltage separation.

Figure 2.7 High-Resolution Shutdown Method (IgG HR Shutdown - PA 800 *plus*.met)



	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								

Figure 2.8 High-Speed Shutdown Method (IgG HS Shutdown - PA 800 *plus*.met)



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	10.00 min	BI:D1	BO:D1	reverse	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	reverse	0.1 N HCl rinse to neutralize capillary surface
3		Rinse - Pressure	50.0 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 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, normal polarity, both	SDS Gel for voltage equilibration
6	10.00	Wait		0.00 min	BI:A1	BO:A1		ddH2O, use for capillary dip to prevent capillary from drying
7	10.00	Lamp - Off						
8								

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