Automated and quantitative analysis of biologics.

PA 800 *plus* Pharmaceutical Analysis System

Now sold through SCIEX Separations
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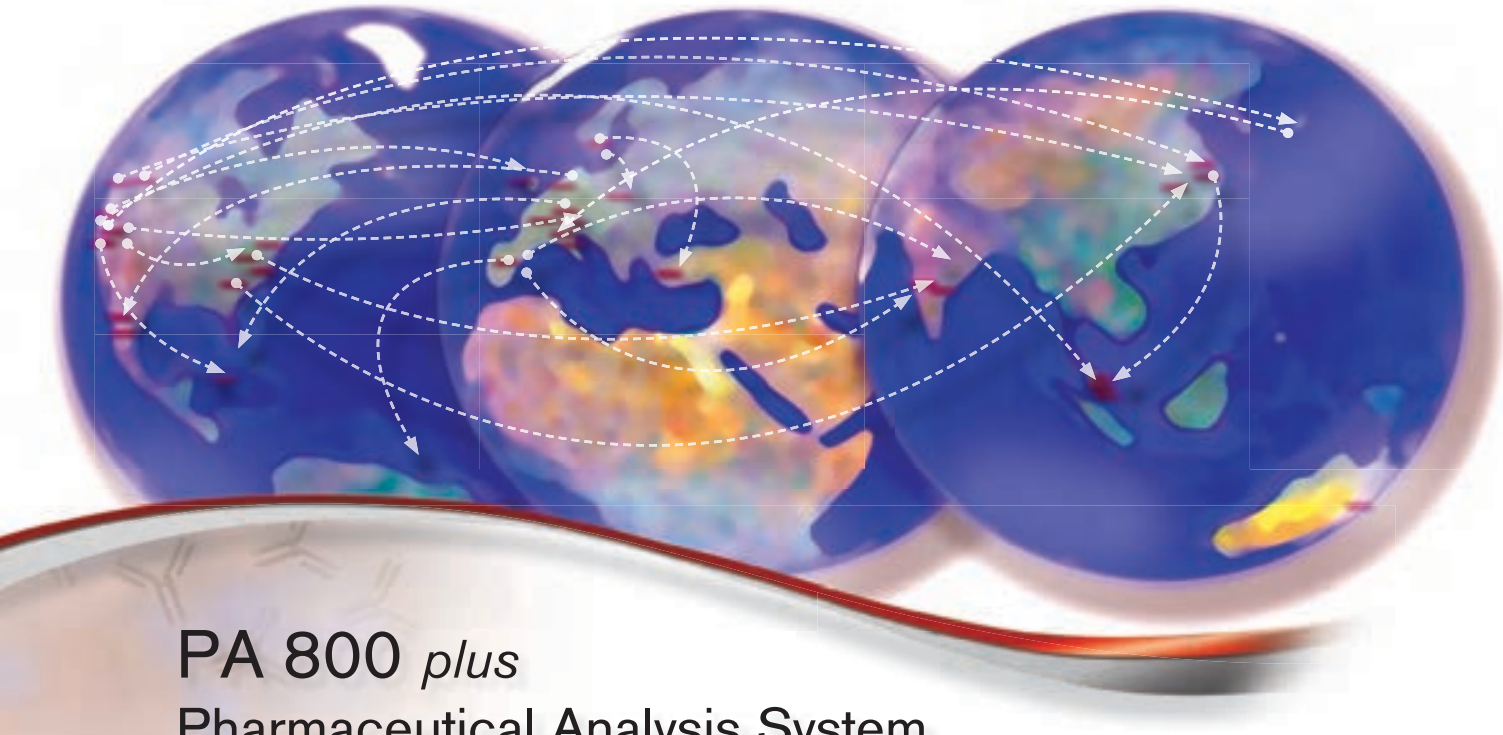
BR-12431D



PA 800 *plus*
Pharmaceutical Analysis System

Designed for the Needs of the Biopharmaceutical Industry

Therapeutic proteins make up a rapidly growing segment of global pharmaceutical production. These complex molecules require accurate characterization of product purity, heterogeneity and identity. This includes data regarding their stability, shelf life and related manufacturing processes.

Analysts handling therapeutic proteins need:

- Automated, qualitative and quantitative analysis
- Simplified functionality and maximum operational efficiency
- Robust, validated applications that can be transferred globally

The PA 800 *plus* Pharmaceutical Analysis System addresses these needs by providing robust analytical tools for the development and quality control of therapeutic proteins.

Automated and Quantitative Analysis of Biologics

The PA 800 *plus* Pharmaceutical Analysis System was designed in collaboration with biopharmaceutical development and QC groups. This platform provides analysts with robust and easy-to-use characterization, integrating quantitative, qualitative and automated solutions for protein purity, charge isoform distribution and glycan analysis. During the design of the PA 800 *plus*, Beckman Coulter emphasized assay portability, enhancing the overall system utility in multi-user, multi-instrument facilities.

Automated applications provide reproducible and quantitative results:

- High-resolution SDS-gel separation for protein purity determination
- Advanced capillary Isoelectric Focusing (cIEF) for charge heterogeneity analysis
- Carbohydrate profiling for assessment of glycoprotein microheterogeneity

Simplified operation and a robust platform enhance operational efficiency:

- Specialized software quickly guides routine users from set-up through results
- Innovations in system design ensure dependable operation and durability, with minimal maintenance
- Modular UV, photodiode array and laser-induced fluorescence detectors can be easily interchanged

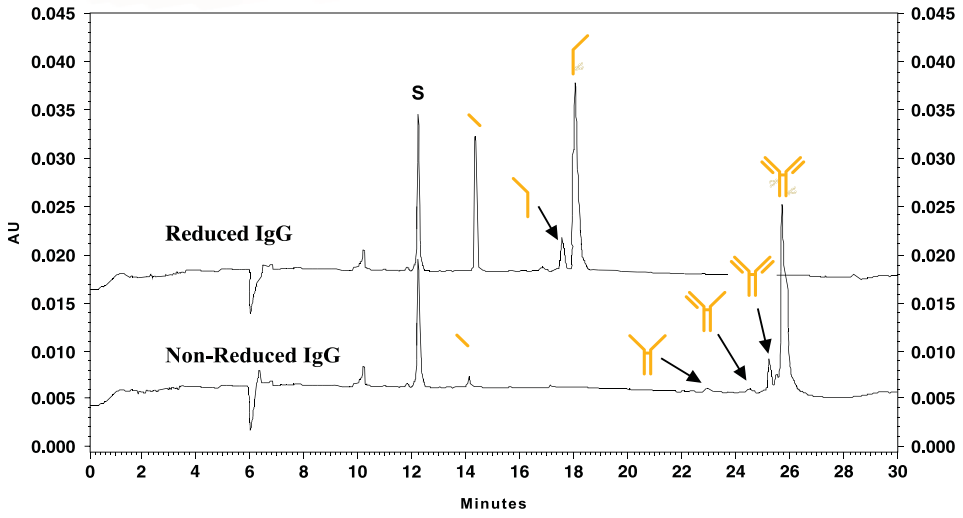
The PA 800 *plus*
Pharmaceutical Analysis System

Applications

PROTEIN PURITY
CHARGE HETEROGENEITY
CARBOHYDRATE PROFILING

Quantitative Protein Purity Analysis with SDS-Gel Capillary Electrophoresis

The PA 800 *plus* Pharmaceutical Analysis System automates size separation of proteins and provides high-resolution, quantitative data, as compared to traditional labor-intensive slab gels. The CE SDS-gel application has become the gold standard for protein purity analysis in biopharmaceutical laboratories. Denatured proteins can be reduced or left intact for separation and subsequent analysis.



The IgG Purity and Heterogeneity Assay provides high-resolution separation for either reduced or non-reduced IgG molecules.

Beckman Coulter's patented replaceable SDS-gel[1] consists of a polymer matrix that allows for:

- Quantitative and automated separation of proteins from 10-225kD
- Sensitivity equivalent to silver-stained gels when using laser-induced fluorescence (LIF) detection
- High-resolution separation capability

1. See US Patent # US7,381,317. Automating the process of gel replacement is disclosed in US Patent # USRE37,606.

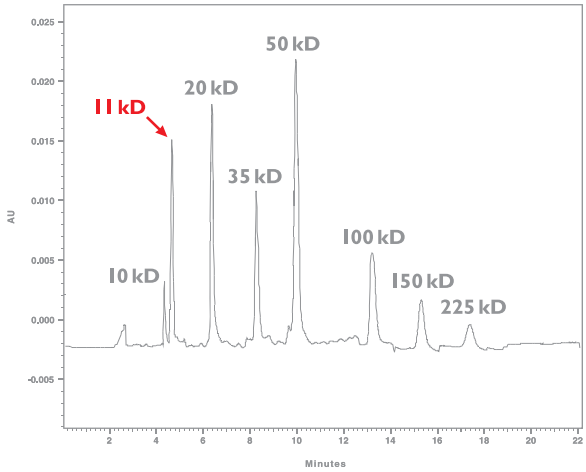
IgG Purity and Heterogeneity Assay

The PA 800 *plus* IgG Purity and Heterogeneity Assay Kit (p/n A10663) was designed in collaboration with biopharmaceutical analysts developing and manufacturing therapeutic MAb molecules. Assay methodology involves heat denaturation of IgG in the presence of SDS, followed by size separation using high-resolution capillary gel electrophoresis technology.

- Detection of impurities below 0.1%
- Repeatability of IgG mobility <1% RSD

The IgG assay on the PA 800 *plus* features an internal system suitability control consisting of an IgG control standard with a designated quantity of non-glycosylated heavy chain to test both the resolution and quantitation suitability of the assay prior to running unknowns.

More information on the SDS-gel application is available in the following Beckman Coulter application information bulletin: "Assay of IgG Purity and Heterogeneity Using High-Resolution Sodium Dodecyl Sulfate Capillary Gel Electrophoresis" (AIB A-1973A).



Beckman Coulter's SDS-gel provides separations across a broad MW range. This specially formulated gel lets you separate small size differences with excellent resolution. The image above illustrates separation of a set of MW standards spiked with an 11kD peptide.

The table below summarizes the results of 18 consecutive SDS-gel analyses of a reduced mouse IgG standard. The relative standard deviation (% RSD) of both the light chain and heavy chain mobility was < 1%, while the quantitative determination of the % light chain (LC), heavy chain (HC) and non-glycosylated heavy chain (NGHC) was also < 1%. Resolution between the NGHC and HC was >1.

Injection	%Corrected Area Reproducibility			Resolution	Mobility Reproducibility	
ID	LC	NG	HC	NG/HC	LC	HC
Injection1	27.90	5.95	49.89	1.42	-0.00004450	-0.00003467
Injection2	27.94	5.96	49.87	1.41	-0.00004450	-0.00003465
Injection3	27.90	5.93	49.94	1.44	-0.00004450	-0.00003464
Injection4	27.96	5.94	49.87	1.41	-0.00004448	-0.00003466
Injection5	27.91	5.93	49.81	1.41	-0.00004452	-0.00003471
Injection6	27.96	5.92	49.87	1.41	-0.00004451	-0.00003468
Injection7	28.01	5.90	49.86	1.40	-0.00004451	-0.00003467
Injection8	27.90	5.93	49.98	1.40	-0.00004450	-0.00003469
Injection9	27.98	5.95	49.84	1.43	-0.00004454	-0.00003472
Injection10	27.94	5.93	49.87	1.43	-0.00004453	-0.00003474
Injection11	28.00	5.93	49.80	1.40	-0.00004456	-0.00003480
Injection12	28.00	5.92	49.76	1.40	-0.00004458	-0.00003482
Injection13	27.97	5.93	49.85	1.40	-0.00004463	-0.00003494
Injection14	28.04	5.90	49.82	1.40	-0.00004466	-0.00003498
Injection15	28.01	5.92	49.76	1.43	-0.00004468	-0.00003500
Injection16	28.03	5.91	49.73	1.43	-0.00004466	-0.00003500
Injection17	28.04	5.91	49.79	1.42	-0.00004470	-0.00003504
Injection18	28.13	5.87	49.70	1.42	-0.00004469	-0.00003506
Min:	27.90	5.87	49.70	1.40	-0.00004470	-0.00003506
Max:	28.13	5.96	49.98	1.44	-0.00004448	-0.00003464
Mean:	27.98	5.92	49.83	1.41	-0.00004457	-0.00003480
Std Dev:	0.06	0.02	0.07	0.01	0.00000008	0.00000015
%RSD:	0.22	0.36	0.14	0.95	0.17	0.44

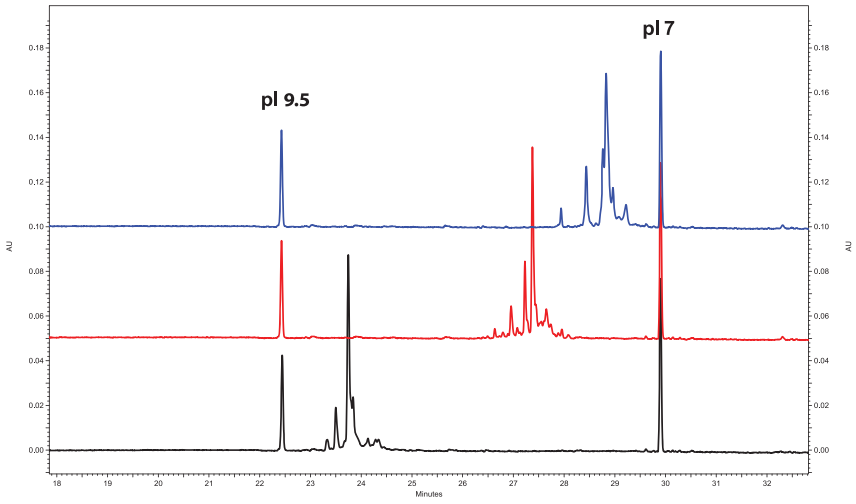
This SDS-gel based method has been successfully implemented by different organizations in different locations with high precision.[2] The portability of the IgG Purity and Heterogeneity Assay was demonstrated in a 2006 study featuring multiple biopharmaceutical companies.

2. "A Series of Collaborations between Various Pharmaceutical Companies and Regulatory Authorities Concerning the Analysis of Biomolecules Using Capillary Electrophoresis." *Chromatographia* 2006, 64, September (No. 5/6).

Photodiode array detection lets you perform high-sensitivity analyses across a broad range of wavelengths. Photodiode array detection between 190 and 600 nm allows for baseline subtraction and spectral wavelength analyses.

Quantitative Protein Charge Heterogeneity

Accurate determination of a protein's charge heterogeneity helps establish identity and stability. Capillary Isoelectric Focusing (cIEF) is a powerful technique that allows quantitative analysis of proteins separated by isoelectric point (pI). The PA 800 *plus* Pharmaceutical Analysis System automates advanced cIEF technology to achieve high precision and quantitative separations. Use of optimized methods and synthetic pI markers attains the highest levels of precision in pI estimation and direct isoform quantitation.



The cIEF application on the PA 800 *plus* has been optimized to provide a single separation method for multiple MAb molecules. In the figure above, three different therapeutic IgG molecules are highly resolved using the same separation conditions.

The following Beckman Coulter Application Information Bulletins describe the advanced cIEF methods in detail:

"Identification of System Parameters Critical for High-Performance cIEF" (AIB A-11634A)

"A Robust cIEF Method: Intermediate Precision for the pH 5-7 Range" (AIB A-12015A)

"High-Resolution cIEF of Therapeutic Monoclonal Antibodies: A Platform Method Covering pH 4-10" (AIB A-12026A)

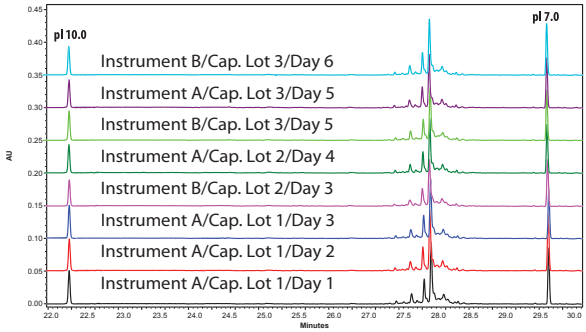
In cIEF, a mixture of sample and ampholyte is introduced into a capillary and subjected to electrophoretic separation. In this process, a pH gradient through which analytes migrate to their respective pI is formed. Comprehensive optimization of multiple assay parameters has been performed.

Successful transfer and implementation of characterization assays between laboratories is based on a method's ability to minimize environmental and operator variability.

An important indicator of the necessary robustness is intermediate precision. Performing advanced cIEF on the PA 800 ***plus*** system provides:

- Intermediate precision for calculated pI at <0.1% RSD
- Intermediate precision for major isoform quantitation at <3% RSD

Beckman Coulter's cIEF Peptide Marker Kit features synthetic peptides. The combination of the advanced cIEF methods, synthetic pI markers and the PA 800 *plus* system results in cIEF separations with the highest level of precision for pI determination and isoform quantitation of your sample.



Intermediate precision for a therapeutic IgG. Quantitative data is shown in the table below.

n = 25

Estimated pI

Peaks	Average	Std Dev	% CV
A	8.31	0.00	0.06%
B	8.18	0.01	0.07%
C	8.13	0.01	0.07%
D	8.07	0.01	0.07%
E	8.01	0.01	0.07%
F	7.90	0.01	0.07%
G	7.78	0.00	0.05%

Isoform Group Percent Composition

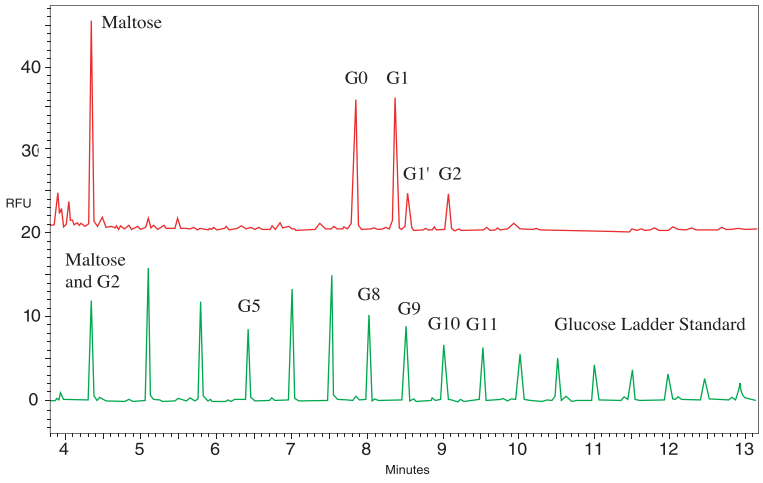
Group	Average	Std Dev	% CV
Basic	30.97%	0.67%	2.17%
Main	45.01%	0.45%	0.99%
Acidic	24.02%	0.60%	2.50%

The table above summarizes the results of a cIEF intermediate precision study on a therapeutic IgG containing 7 isoforms. This MAb was separated in triplicate on two different instruments using three different lots of neutral capillary and reagents on 6 different days. Isoforms were grouped as acidic, main or basic and then analyzed. The coefficient of variance (% CV) for each of the 7 peaks was < 1%, while the quantitative determination of the isoform group % composition was < 3%. The main isoform group % composition was < 3%.

The PA 800 *plus* UV/Vis Detection Module provides absorbance spectroscopy in the UV-visible region. Commonly used exclusion filters at 200 nm, 214 nm, 254 nm and 280 nm are provided to increase analyte specificity.

Carbohydrate Profiling and Analysis for Microheterogeneity Determination

Glycosylation on a protein is an important post-translational modification that can affect its function, clearance and stability. The PA 800 *plus* Pharmaceutical Analysis System simplifies the complex process of profiling carbohydrates associated with glycoproteins. By providing specific and accurate quantitation of glycosylation levels, differences in glycoform quantities and distribution can be determined.



Detection of *N*-linked oligosaccharides isolated from mouse IgG2 molecule yields a separation capable of differentiating positional isomers G1 and G1'. An APTS-labeled glucose ladder is also shown.

Key benefits to carbohydrate profiling using capillary electrophoresis include:

- Quantitation of *N*-linked oligosaccharides[3]
- High-resolution separation methods capable of differentiating positional isomers
- Validated methodology developed for routine use environments

3. APTS-labeled carbohydrate analysis using capillary electrophoresis is a patented technology (US Patent # US5,569,366).

Oligosaccharide Analysis Simplified

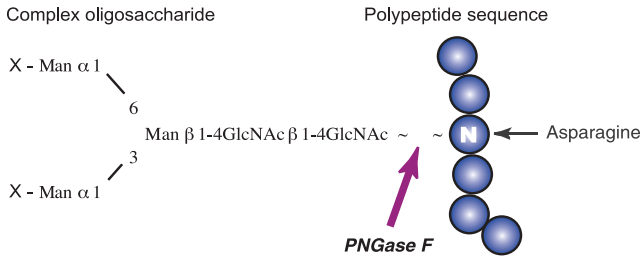
Following endonuclease cleavage from the protein, oligosaccharides are specifically labeled with amino-pyrene-trisulfonic acid (APTS) by reductive amination. This analysis is performed directly from the glycoprotein hydrolysate.

With APTS derivatization and LIF detection, each sugar yields the same detector response, so their relative quantities can be directly compared.

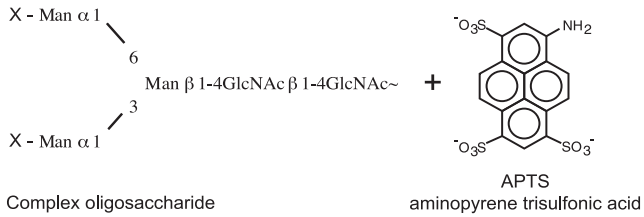
Oligosaccharide distribution associated with a protein yields a fingerprint that can be used in protein identification. The PA 800 ***plus*** provides easy, quantitative and robust determination of protein microheterogeneity, with a typical analysis time of less than 15 minutes.

The Carbohydrate Labeling and Analysis Assay is described in more detail in the following Beckman Coulter application information bulletin: "CE Separation of ***N***-Linked Oligosaccharides Released from Recombinant Monoclonal Antibody" (AIB A-1986A).

1. N-Glycosidase Cleavage



2. APTS Labeling



The 488 nm solid state laser module provides robust fluorescence technology in a quiet, energy-efficient and compact design.

Software as Easy as 1,2,3

1.

Select application

2.

Load samples and application reagents

The PA 800 *plus* software quickly guides users from set-up through routine system operation. Large icons provide intuitive guidance for navigation, while on-screen cues indicate system progress at a glance. Insightful Help menus and descriptive system prompts further simplify operator learning, making transfer of PA 800 *plus* technology to other analysts easier. Ultimately, using PA 800 *plus* software is as easy as 1, 2, 3.

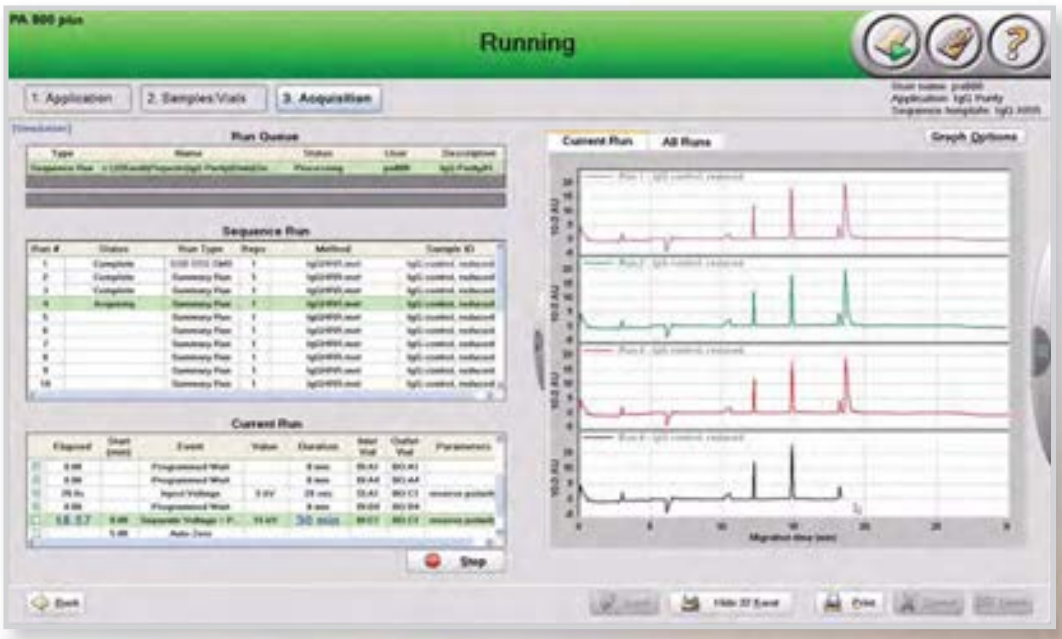
Use the Help and video menu as needed.

PA 800 *plus* software features include:

- Automated sequence table and reagent calculations
- Validated applications for SDS-gel, cIEF and carbohydrate analysis
- Enhanced Help menu and instructional videos
- Technical controls enabling regulatory compliance
- On-screen prompts for monitoring system events
- Advanced reporting capability
- Increased workspace

3.

Acquire data



A Robust Solution for Demanding Research Requirements

The PA 800 *plus* offers dependable, accurate determination of protein purity, heterogeneity and identity. To create it, Beckman Coulter collaborated with biopharmaceutical development and QC groups experienced in the routine use of capillary electrophoresis for protein characterization.

The universal vial and cap design prevents the capillary and electrode from physically interacting with the caps, ultimately allowing for a robust, clean sample interface.

Automated Sample Introduction

The PA 800 *plus* system offers fully automated methods and extended sample-handling capability for walk-away operation. Sampling can be performed using 1.8 ml universal vials, 96-well plates and micro vials. Precision-molded polymethylpentene universal vials accommodate run buffer, sample and micro vials.

Sample Temperature Control

Sample temperature control maintains molecular stability when working with temperature-labile protein species. The sample temperature can be maintained between 4 - 60°C.

Temperature Control of the Capillary

Efficient separations in CE rely on precise regulation of the capillary temperature to manage Joule heating within the capillary. Proper temperature control plays an important role in the repeatability of SDS-gel, cIEF and carbohydrate analysis.

The PA 800 *plus* uses recirculating liquid coolant to provide effective heat dissipation when performing assays on the system. Capillaries are housed in patented cartridges, facilitating both temperature control and easy exchange of capillary dimensions and surfaces (US Patent # 5198091; see application bulletin T1823ab). Capillary temperature can be regulated between 15 - 60°C.

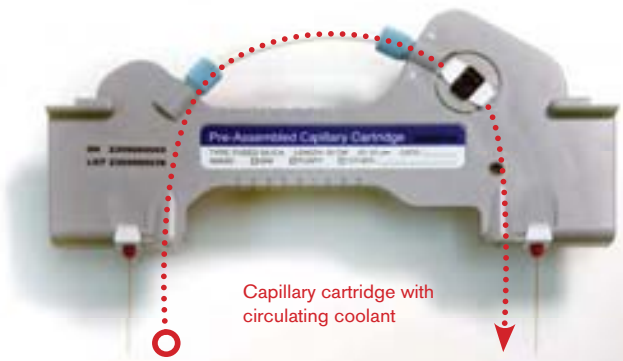
The integrated, solid state 488 nm laser reduces the overall system footprint.

Multiple Modes of Sample Introduction and Separation

The PA 800 *plus* offers electrokinetic, pressure and vacuum injection of samples. Additionally, injection from either end of the capillary allows both ultra-fast and high-resolution analyses. Separations can be adjusted by varying voltage, current, pressure and vacuum. The combination of voltage and pressure in the SDS-gel assay ensures the gel buffer stays free of air bubbles which can be generated from gel outgassing.

Variable Pressure and Vacuum

The PA 800 *plus* operates with all common rinsing protocols, regulating them with a pressure-handling capability of -5 to 100 p.s.i. Capillary conditioning is accomplished by moving specific volumes of electrolytes, gels, regenerants and cleaning solutions through the capillary. Gel buffers are quickly and efficiently pumped into the capillary.



Capillary cartridge with circulating coolant

Versatile Modular Detection Capability

Each PA 800 *plus* offers precise, real-time analysis for a variety of assays, because it integrates UV, photodiode array and LIF detection capabilities in one unit.

UV detection is important when using photosensitive capillary surfaces. Photodiode array detection between 190 and 600 nm allows for baseline subtraction and spectral wavelength analyses. A 488 nm solid state laser and laser-induced fluorescence (LIF) detector permits high-sensitivity analysis of labeled molecular species.

CE-MS Ready

Capillary electrophoresis separation coupled to mass spectrometry (MS) combines the high-resolution separation of CE and the high-sensitivity mass determination of MS. The PA 800 *plus* accommodates direct MS connection through the right-side access panel. Capillary temperature control is maintained.

Supplies and Resources

Reagents	
SDS-MW Assay Kit	390953
SDS-Gel Multipack (4 bottles)	A30341
IgG Purity and Heterogeneity Assay	A10663
10 kD Protein Standard	A26487
IgG Control Standard (3-pack)	391734
MW Sizing Standard	A22196
Carbohydrate Labeling and Analysis Assay	477600
cIEF Peptide Marker Kit (pI Marker Kit)	A58481
Neutral Capillary	477441
N-CHO Capillary	477601
Bare Fused Silica Capillaries (3)	338451
Advanced cIEF Starter Kit	A80976

Supplies and Accessories	
Universal Vials	A62251
200 µL Microvials (pkg of 100)	144709
Universal Vial Caps	A62250
Electrode Replacement Kit	A47775
Vial Cap Opener	A95348
Buffer Vial Tray (36 vials)	A58254
Buffer Vial Tray (48 vials)	A58255
Cartridge Assembly, 30 cm Capillary	A11147
Blank Cartridge Assembly Kit	144738
Cartridge Rebuilding Kit	144645
Cartridge Tubing Kit	144689
Capillary Coolant (450 mL)	359976
Cartridge with MS Adaptor	A61216

The items can be ordered at
www.beckmancoulter.com/PA800plus

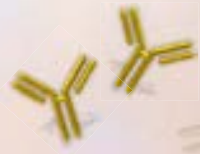
Ordering Information

A66528 PA 800 *plus* Pharmaceutical Analysis System

Includes separation module with UV, photodiode array and LIF detection; system controller with PA 800 ***plus*** software; system startup kit and reagents

A66527 PA 800S *plus* Pharmaceutical Analysis System

Includes separation module with photodiode array detection; system controller with PA 800 ***plus*** software; system startup kit and reagents



System Specifications

Dimensions:
- Height: 29.2 inches (74.2 cm)
- Door Open: 38.8 inches (98.6 cm)
- Width: 25 inches (63.5 cm)
- Depth: 28.4 inches (72.1 cm)

Weight (Uncrated):
- 188 lbs (85.3 kg)
- (includes photodiode array detection)

Electrical Requirements:
- Voltage: 100 - 240V 50/60Hz

Voltage Range:
- 1 to 30 kV programmable at
- 0.1 kV increments

Current Range:
- 3 to 300 µA programmable at
- 0.1 µA increments

Pressure Delivery Range:
- -5 to +100 psi

Sample Temperature Control:
- 4 - 60°C

Capillary Temperature Control:
- 15 - 60°C

System Capacity

Sample Trays:
- 2 x 96-well plates
- 2 x 48 universal vials
- 2 x 48 0.3 ml vials or microfuge tubes

Buffer Tray:
- 2 x 36 universal vials

Detection Capability:
- UV/Vis
  - 200, 214, 254, 280 nm standard filter
  - 190 - 600 nm (custom filter option)
- Diode Array
  - 190 - 600 nm (programmable)
  - 0.5 - 32 Hz scan collection frequency (programmable)
- Laser Induced Fluorescence (LIF)
  - 300 - 700 nm excitation range
  - 350 - 750 nm emission range
  - 0 - 1000 RFU

Source Lasers with 3 mW Power Output:
- 488 nm solid-state laser
- (included in A66528)
- 635 nm diode laser (optional)

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