Automated and Quantitative Analysis of Biologics

PA 800 PLUS PHARMACEUTICAL ANALYSIS SYSTEM

For Research Use Only. Not for use in diagnostic procedures.
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.

Research Analysts handling therapeutic proteins need:

- Automated, qualitative and quantitative analysis
- Simplified functionality and maximum operational efficiency
- Limitless flexibility for method development
- Simple enough for routine use yet includes system controls for full method optimization
- Robust, validated applications that can be transferred globally

The PA 800 Plus Pharmaceutical Analysis System enables all of these needs, providing a robust analytical platform with easy-to-use software for the development and quality control of therapeutic proteins.

Automated and quantitative analysis of biologics

The PA 800 Plus Pharmaceutical Analysis System has been adopted by many companies in the biopharmaceutical industry and global regulatory organizations. The assays we pioneered for purity and heterogeneity determination for use on this platform are referenced in pharmacopoeial methods. Analysts benefit from quantitative, qualitative, and automated protein purity, charge isoform distribution, and glycan analyses. The PA 800 Plus assays below have proven portability and enhance the overall system utility in global multi-user, multi-instrument organizations.

- High-resolution SDS gel protein separations with up to 4 orders of magnitude in detection
- Advanced capillary isoelectric focusing (cIEF) and high speed capillary zone electrophoresis (CZE) for charge heterogeneity analysis
- Carbohydrate profiling for assessment of glycoprotein microheterogeneity


Multi-sites N-glycan mapping study 1: Capillary electrophoresis – laser induced fluorescence, mAbs. Volume 6, Issue 6, 2014

Intercompany Study to Evaluate the Robustness of Capillary Isoelectric Focusing Technology for the Analysis of Monoclonal Antibodies. Chromatographia (2011) 73:1137-1144
Simplified operation on a robust platform enhances operational efficiency:

- Specialized software quickly guides routine users from set-up through results
- Ready-to-use cartridge for purity assessment ensures data consistency and ease of use
- Innovations in system design provide dependable operation and durability, with minimal maintenance
- Modular UV, photodiode array and laser-induced fluorescence detectors can be easily interchanged
- Methods can be locked down in Analytical Development for routine use in QA/QC
High-resolution, quantitative data

The PA 800 Plus Pharmaceutical Analysis System automates size separation of proteins and provides high-resolution, quantitative data. The CE SDS-gel application has become the gold standard for protein purity analysis in biopharmaceutical laboratories, replacing manual, low resolution SDS-PAGE. Denatured proteins can be reduced or left intact for separation and subsequent analysis.

Replaceable SDS-gel\(^2\) consists of a polymer matrix that allows for:

- High-resolution separation capability
- Quantitative and automated separation of proteins from 10 - 225 kDa
- Sensitivity equivalent to silver-stained gels when using laser-induced fluorescence (LIF) detection
- Modular UV/vis and LIF detection allow up to 4 logs of detection

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.

- Impurity detection to 0.01% using LIF and 3 orders of magnitude when using UV detection
- 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.

The United States Pharmacopeial Convention has included IgG Purity and Heterogeneity analysis in Chapter <129> - Analytical Procedures for Recombinant Therapeutic Antibodies (source: United States Pharmacopeial Convention; usp.org)

Understanding the Separation Matrix

It's important to understand the components making up your CE separation matrix. Our replaceable CE-SDS gel was developed for use in the IgG assay on the PA 800 Plus. The core elements include a dextran polymer in tris borate buffer pH 8.0 – 8.3, Glycerol added to increase the molecular size separation range, and EDTA to minimize protein oxidation brought about by metal ion chelation. This formulation has proven\(^1\) to be an excellent matrix in which to resolve even the smallest difference in migration time for an increased number of therapeutic proteins.

Analysis of both the reduced and non-reduced IgG Suitability standard using the IgG purity method. Peak 1: Internal standard (10 kDa); 2: Light Chain (L); 3: NG heavy chain; 4: Heavy chain (H); 6: Heavy-heavy chain (2H); 7: 2 heavy 1 light chain (2H1L); 8

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.

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

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.

<table>
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<tr>
<th>Injection</th>
<th>%Corrected Area Reproducibility</th>
<th>Resolution</th>
<th>Mobility Reproducibility</th>
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<tr>
<td>ID</td>
<td>LC</td>
<td>NG</td>
<td>HC</td>
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<tr>
<td>Injection1</td>
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<td>%RSD:</td>
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</table>

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

“This exercise supports and endorses the increased application of CE methodology within both development and QC laboratories within biopharmaceutical companies.”2

High precision and quantitative separations

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). Capillary Zone Electrophoresis (CZE) provides high speed charge heterogeneity analysis using simple sample preparation.

The PA 800 Plus Pharmaceutical Analysis System automates advanced cIEF and CZE technologies to achieve high precision and quantitative technologies separations. Use of optimized methods and synthetic pI markers in cIEF attains a high level of precision in pI cIEF estimation and direct isoform quantitation.

High Throughput and Ultra High Resolution Options

“CZE appears as a new powerful platform technology for the charge heterogeneity testing of antibodies in the pharmaceutical industry.”

Comparison between cIEF and CZE of a therapeutic MAb.

Whereas cIEF provides ultra-high resolution between charge isoforms, CZE combines less complex sample preparation and faster separations without significant loss in resolution.

CZE = mobility-based
- charge
- hydrodynamic size

cIEF = charge-based

Application information bulletins describing the advanced cIEF and CZE 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)
- “Analysis of Monoclonal Antibody Charge Variants by CZE” (IB-17031A)

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. Requiring less sample preparation than cIEF, CZE generates charge isoform heterogeneity data fast and with very high resolution.
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.

The table 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%.

<table>
<thead>
<tr>
<th>N = 25</th>
<th>Estimated pl</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>A</td>
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<td>C</td>
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<td>D</td>
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<tr>
<td>E</td>
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<td>F</td>
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<td>G</td>
<td>7.78</td>
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<table>
<thead>
<tr>
<th>Isoform Group Percent Composition</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>Basic</td>
</tr>
<tr>
<td>Main</td>
</tr>
<tr>
<td>Acidic</td>
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References for above referenced publications:
(1) Salas-Solano O et al. (2011) Intercompany Study to Evaluate the Robustness of Capillary Isoelectric Focusing Technology for the Analysis of Monoclonal Antibodies. Chromatographia 73:1137-1144
(2) Moritz B et al. J Chromatography B. article in press.

and reproducibility. Inter-company collaborative studies illustrating assay robustness and portability have been performed in the biopharmaceutical industry for both cIEF and CZE.

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 and CZE on the PA 800 Plus system provides:
• Repeatability of IgG mobility <1% RSD
• Detection of impurities below 0.1%
Key benefits to carbohydrate profiling using capillary electrophoresis include:

- Quantitation of N-linked oligosaccharides
- High-resolution separation methods capable of differentiating positional isomers
- Validated methodology developed for routine use environments
- Data can be used with Glycobase for easy identification of glycan species.

Oligosaccharide analysis

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.


Fast, Reproducible, High Resolution Glycan Analysis

Simplified processing

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.

Rapid Glycan Analysis:

- 1 hour sample prep
- 3 min. separation per sample
- Data in <6 hours for 96 samples
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 and with method optimization as fast as 3 minutes per separation without significant loss in resolution.

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

View the video: Sample preparation of mAb N-glycans using Magnetic Bead Technology and CE-LIF.

More information is available in the following application bulletin: Separation of Fucosylated, non-Fucosylated, and Complex Carbohydrates Associated with Monoclonal Antibodies using Capillary Electrophoresis (AIB-15285A)

The applied CE-LIF method for the analysis of glycans provides reliable intra- and inter-laboratory results across laboratories and it is therefore a robust tool for N-glycan analysis that can confidently be applied on its own or as an orthogonal technique to other existing methods.

The United States Pharmacopeial Convention has included glycan analysis by CE for determination of IgG Microheterogeneity analysis in Chapter <129> Analytical Procedures for Recombinant Therapeutic Antibodies. (source: United States Pharmacopeial Convention; usp.org)
Incorporating tools to enable 21 CFR part 11 compliance, 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.
3 Acquire data

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 enable regulatory compliance
- On-screen prompts for monitoring system events
- Advanced reporting capability
- Increased workspace

Use the Help and video menu as needed.
A Robust Solution for Demanding Research Requirements

The PA 800 Plus offers dependable, accurate determination of protein purity, heterogeneity and identity. Close collaboration with biopharmaceutical development and QC groups experienced in biologics characterization helped create assays which address analytical challenges.

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, independent from buffers, enables users to maintain molecular stability when working with temperature-labile protein species. The sample temperature can be between 4 - 60°C.

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.

Temperature Control of the Separation 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 cartridges facilitating both temperature control and easy exchange of capillary dimensions and surfaces (see application bulletin T1823ab). Capillary temperature can be regulated between 15 - 60°C.

nanoVial
Multiple injections from 5 μL sample amount can be performed with the nanoVial.
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.

Ready-to-Use Capillary Cartridges

CE-SDS can be performed using factory manufactured cartridges, ready-to-use with bare fused-silica capillaries. Ready-to-use cartridges ensure precision cut ends and alignment of capillary windows with the detection path, resulting in consistency of your separations.

Capillary cartridge with circulating coolant

Versatile Modular Detection Capability

The 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

For high sensitivity applications we recommend the CESI 8000 Plus High Performance Separation - ESI Module. With the CESI 8000, stable spray is achieved at ultra-low flow rates (<10 nl/min) resulting in the following benefits:

- Ion suppression bias is virtually eliminated
- Ionization efficiency maximized giving an overall increase in sensitivity
- With optional detectors the CESI 8000 Plus has all the abilities of the PA 800 Plus as well as MS connectivity.
- No detectable sample carryover

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

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<tr>
<th>Category</th>
<th>Product Description</th>
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<td>Purity</td>
<td>SDS-Gel Multipack (4 bottles)</td>
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<td>IgG Purity and Heterogeneity Assay</td>
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<td>10 kD Protein Standard</td>
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<td>IgG Control Standard (3-pack)</td>
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<td>Bare Fused Silica Capillaries (3)</td>
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### Supplies and Accessories

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<td>Pre-assembled Cartridge (includes bare fused-silica capillary)</td>
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Items can be ordered at [sciex.com/contact-us](http://sciex.com/contact-us)
## System Specifications

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

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

**Electrical Requirements:**
- **Voltage:** 100 - 240 V; 50/60 Hz
- **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 0.2 mL microvials

**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)

## 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
Your Success is Our Success
We take it personally

As a SCIEX customer you have access to a world-class customer support organization. Wherever you are, we’re there with you as a trusted partner to answer questions, provide solutions, and maximize lab productivity.

Our customer support organization has access to the latest product updates, software revisions, methods and repair procedures to make sure that you stay on top of your game.

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