systems

AAA-Direct™ Amino Acid Analysis System

The Dionex AAA-Direct *Amino Acid Analysis System revolutionizes the determination of amino acids. Unlike existing methods, amino acids are detected directly, with high sensitivity, by integrated pulsed amperometric detection (IPAD). Pre- or postcolumn derivatization is not required. The system incorporates a new 2-mm i.d. microbore anion-exchange column, the Dionex AminoPac® PA10, which was designed specifically for high- resolution separation of amino acids.*

The simplicity of the direct detection technique makes it applicable to a wide range of applications in protein characterization and food analysis. In addition, the AAA-Direct *system offers unique advantages for on-line monitoring and optimization of processes such as large-scale cell cultures and fermentation broths used in the production of protein- and peptide-based therapeutics.*

Detection

- Direct detection of primary and secondary amino acids by IPAD. No pre- or postcolumn derivatization required.
- *AAA-Certified*™ Gold electrodes with performance optimized for amino acid analysis.

Separation

- High-resolution separation of amino acids and amino sugars.
- Anion-exchange separation virtually eliminates matrix dependency.
- Amino acid and carbohydrate profiling in a single run.
- Single peak for each amino acid.

Performance

- High sensitivity—mid femtomole to low picomole detection limits. At least 50× more sensitive than ninhydrin-based analyzers.
- Linearity of detector response over three orders of magnitude for most common amino acids.
- Reliable quantitation—good agreement with cation-exchange/ninhydrin-based amino acid analysis methods.
- Straightforward determination of problematic amino acids, for example tryptophan, cysteine, cystine, methionine, and phosphoamino acids.
- Faster and simpler analysis of difficult protein samples (e.g., collagen).
- Minimal sample preparation.
- Compatibility with all commonly used hydrolysis procedures, such as, 6 M HCl, 4 M methanesulfonic acid (MSA), performic acid oxidation/ 6 M HCl, and 4.2 M NaOH.

DIONEX

Instrumentation

- High-performance quaternary gradient microbore pumping system with built-in degasser provides accurate flow, composition control, and low eluent consumption.
- Inert, nonmetallic PEEK (polyether-etherketone) components and flowpath throughout the system ensure compatibility with corrosive eluents and buffers.
- Thermostatted compartment can accommodate two columns, detector cells, and injection valve.
- Sample preparation option automates operations such as simple or serial dilutions for preparation of multilevel calibration standards, and automatic generation of calibration curves.

Economical and Easy Operation

The 2-mm microbore format used in the *AAA-Direct* Amino Acid Analysis system results in low eluent usage, which translates to more convenient operation and time savings. Direct detection by IPAD eliminates the need for expensive reagents and time-consuming sample derivatization procedures. Typically only sample dilution and filtration are required with the *AAA-Direct* system. Cumbersome and maintenance-intensive post-column pumping systems and multiple wavelength monitoring are also unnecessary. A typical chromatogram for a 100-pmol standard is shown in Figure 1.

Excellent Retention Time and Detector Stability

Excellent stability of detector response and retention times has been demonstrated for amino acid standards and real-world samples, including commercial cell cultures, fermentation broths, and protein and peptide hydrolysates. Figure 2 shows a plot of the detector response over 100 consecutive injections of an amino acid standard mixture. Figures 3 and 4 show plots of the reproducibility of retention time and detector response, respectively, for over 100 injections of a cell culture medium sample.

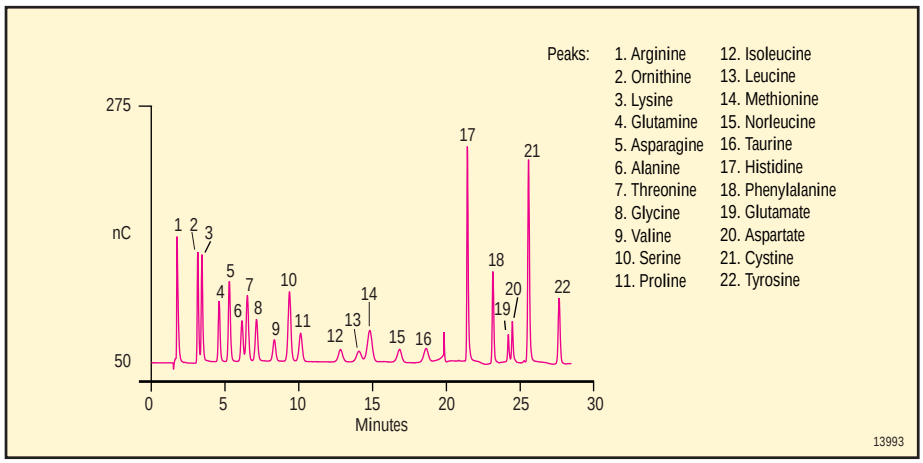

***Figure 1**. Separation of an amino acid standard mixture containing 100 pmol of each component using standard* AAA-Direct *gradient conditions.*

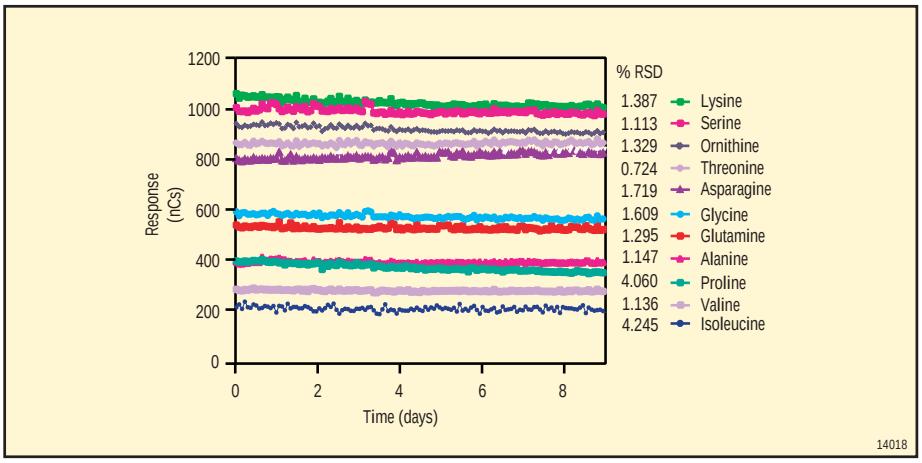

***Figure 2**. Stability of detector response for 100-pmol standard.*

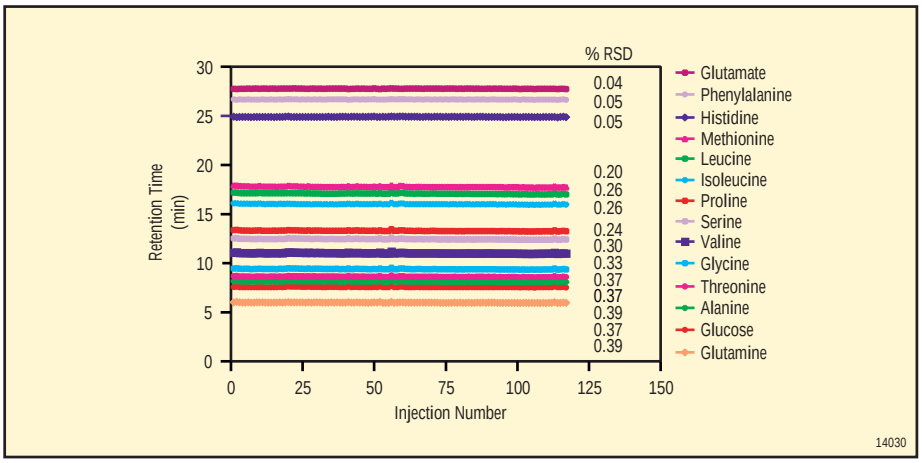

***Figure 3**. Long-term stability of retention time for 1:250 dilution of a cell culture medium sample.*

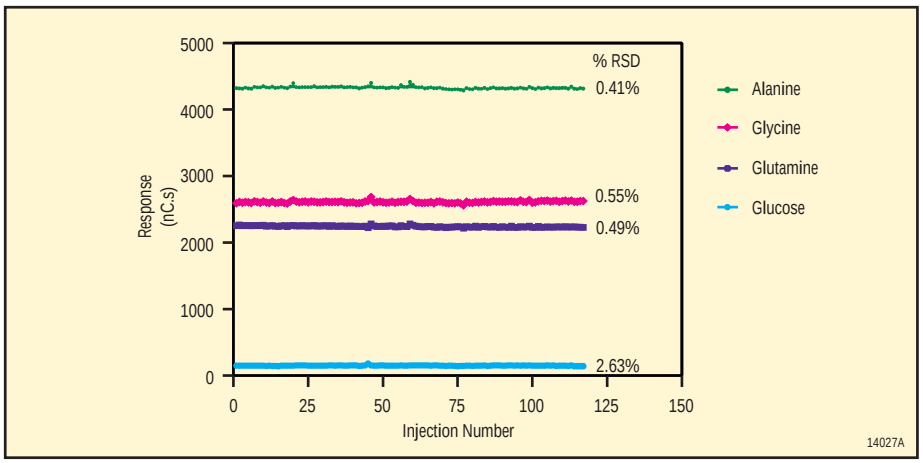

Figure 4*. Long-term stability of response for glutamine and glucose in a cell culture medium sample (1:250 dilution).*

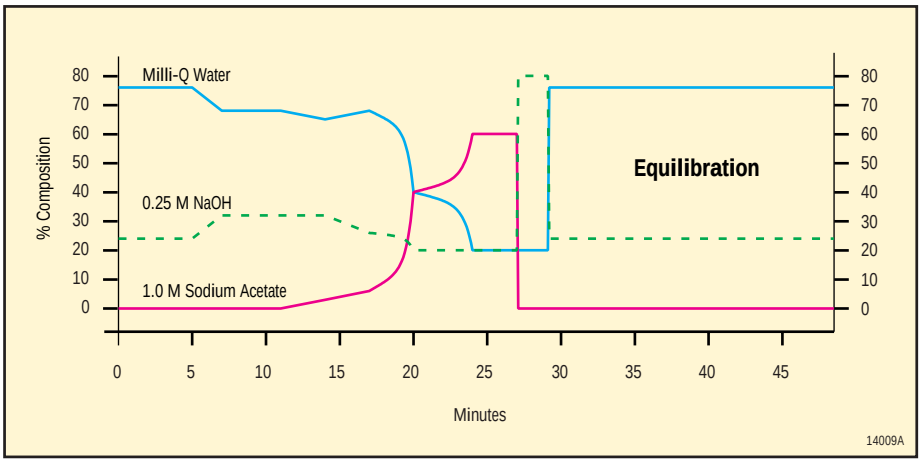

Figure 5*. Standard ternary gradient profile for* AAA-Direct *amino acid analysis.*

Rugged and Reliable Column Technology

The AminoPac PA10 microbore column is a polymer-based high-performance anion-exchange column selectivity optimized for amino acid separations. The MicroBead™ stationary phase is made by coating a nonporous, noncompressible, polymeric substrate with quaternary ammmonium-functionalized latex. This structure results in a highly stable particle with a thin surface layer rich in ion-exchange sites. Advantages of this column technology over conventional ion exchange packings include:

- Rapid reequilibration after gradient elution
- Excellent mass transport characteristics for high efficiency
- Complete pH compatibility (pH 0–14)
- High mechanical (4000 psi) and chemical stability for exceptional column life
- Compatibility with common organic solvents for rapid cleanup after injection of complex matrices

Typical Gradient Program

Figure 5 shows the standard ternary gradient (water: 0.25 M sodium hydroxide: 1.0 M sodium acetate) for the separation of a set of 22 common amino acids. If required, the gradient program can be easily customized for particular applications.

Compatible with All Standard Hydrolysis Procedures

Any of the four common hydrolysis procedures (i.e., 6 M HCl, 4 M MSA, performate oxidation/6 M HCl, and 4.2 M NaOH) can be used with the *AAA-Direct* Amino Acid Analysis System. Also, because the chromatographic retention mechanism is ion exchange, neutral sample components are not retained by the AminoPac PA10 column, virtually eliminating any matrix dependence.

Simplified Tryptophan Determinations

Determination of tryptophan can be problematic due to decomposition under typical HCl hydrolysis conditions. The alternative procedure using NaOH hydrolysis has drawbacks when using pre- or postcolumn derivatization techniques because the hydrolysate must be neutralized and diluted. This procedures requires larger amounts of valuable sample to be used in the case of cation-exchange/ninhydrin-based systems. Interference with the derivatization step by the large amount of NaCl produced can be a problem with precolumn derivatization methods. Using the *AAA-Direct* Amino Acid Analysis System, determination of tryptophan following NaOH hydrolysis is straightforward because an NaOH eluent is used. Sample preparation is greatly simplified because neutralization is unnecessary. Only dilution and filtration of the hydrolysate are required prior to injection.

Single-Run Determination of Amino Acids, Amino Sugars, and Carbohydrates

Amino sugars are often present in protein hydrolysates and can be determined directly along with amino acids because they are well resolved on the AminoPac PA10 column (Figure 6).

In the biotechnology industry, the *AAA-Direct* Amino Acid Analysis System has been used in both benchtop and on-line configurations for direct monitoring of amino acids and sugars in fermentation broths and large-scale cell cultures. This capability provides a simpler approach to monitoring amino acid and carbohydrate nutrients during the production of protein- and peptide-based therapeutics.

Figure 7 shows an analysis of a cell culture. The insert is the IPAD waveform used in the analysis with the integration period indicated by the heavy blue line. Using this waveform, common sugars and amino acids present in the sample can be determined simultaneously. If the integration period is shifted to a lower potential, the response is suppressed for amino acids other than hydroxy amino acids. Carbohydrates and hydroxy-amino acids can then be detected selectively in the same sample.

Figure 8 shows a series of superimposed chromatograms from a time-course study on an *E. coli* fermentation broth. The growth and decay of various amino acid components over time are clearly visible. Optimization of product yield is thus greatly facilitated because the level of essential amino acids can be easily monitored and adjusted as required.

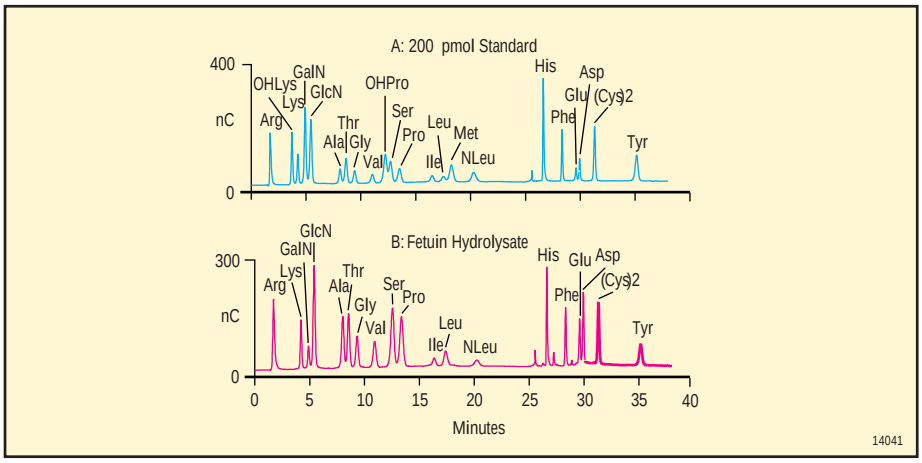

***Figure 6**. The amino sugars galactosamine (Ga1N) and glucosamine (G1cN), which are often present in protein hydrolysates, are well-resolved under the standard* AAA-Direct *gradient conditions as shown here for a fetuin hydrolysate.*

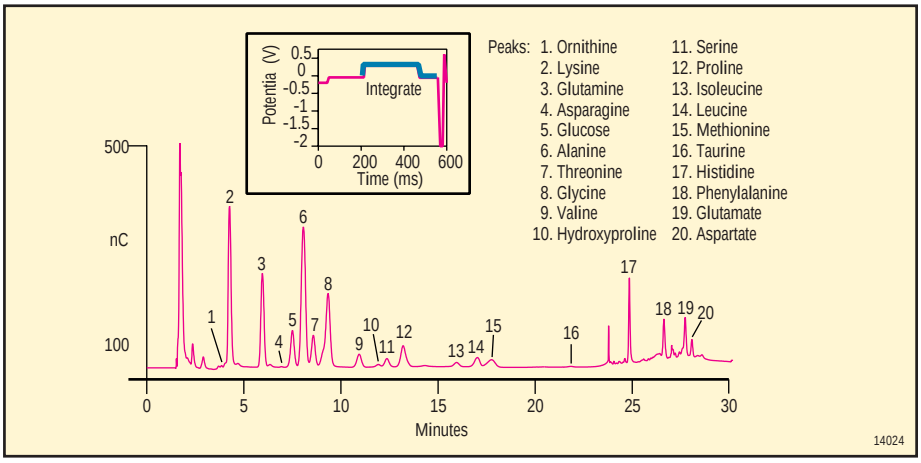

***Figure 7**. Simultaneous analysis of amino acids and glucose in a cell culture (1:250 dilution).*

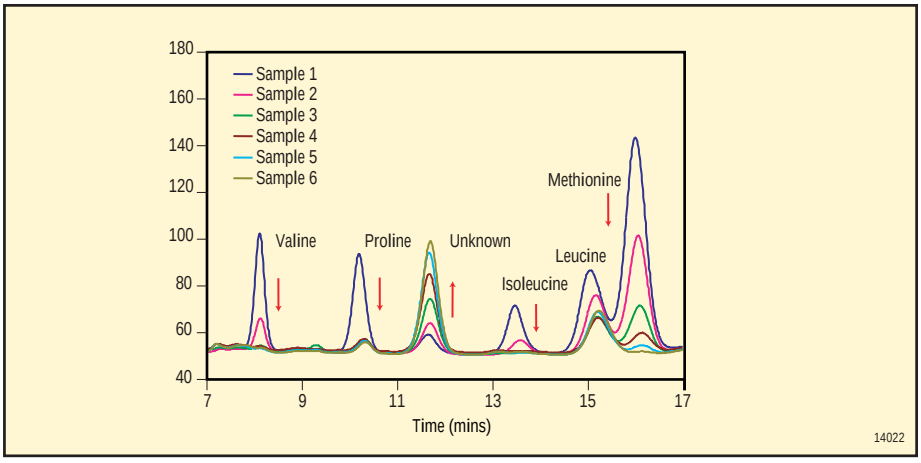

***Figure 8**. Time-course study of amino acid concentrations in an* Escherichia coli *fermentation broth.*

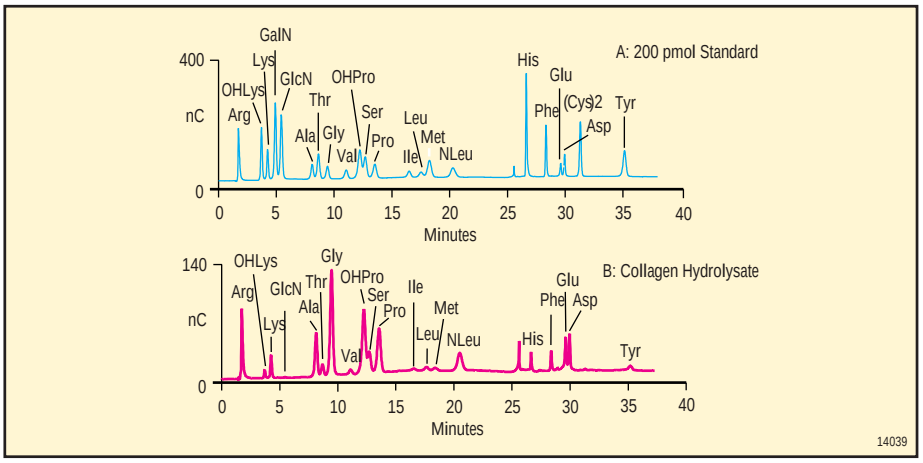

***Figure 9**. Collagen hydrolysate amino acid analysis.*

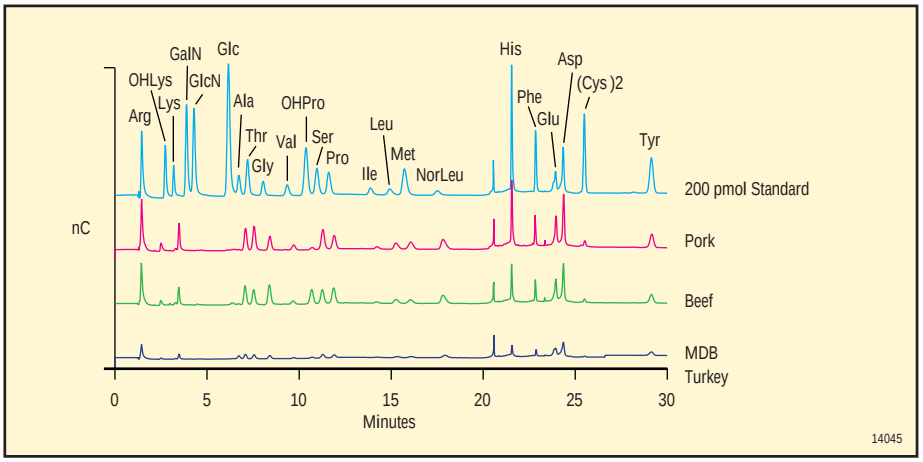

***Figure 10**. Amino acids in unprocessed meat hydrolysates.*

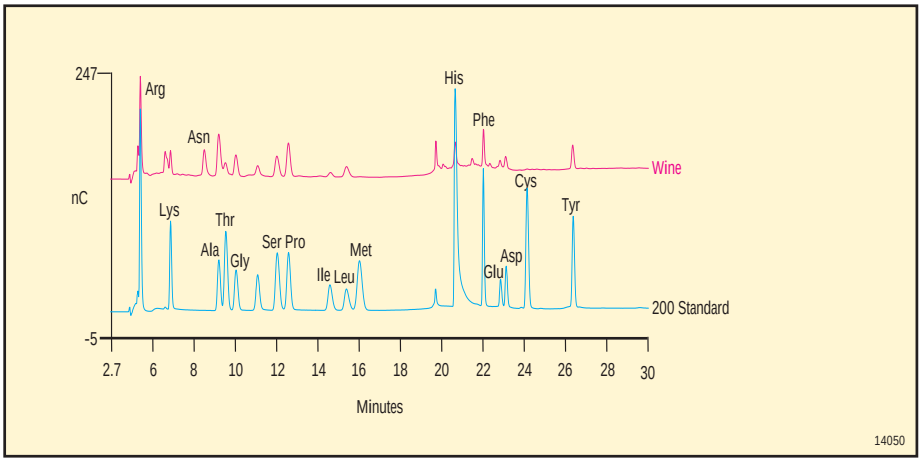

***Figure 11**. Amino acids in rice wine. Carbohydrates were removed from the sample by column switching using Carbohydrate Removal Accessory (CRA) from Dionex.*

Protein and Peptide Hydrolysates

A variety of protein and peptide hydrolysate samples, including collagen, have been successfully analyzed using the *AAA-Direct* system and show good agreement with the postcolumn ninhydrin method. Figure 9 shows a chromatogram for a collagen hydrolysate. Note that only a single injection is required for quantification of all amino acids.

Analysis of Food and Beverage Samples

A variety of food and beverage samples have been analyzed, including unprocessed and processed meat hydrolysates, fruit and vegetable juices, beer, and wine. Figure 10 shows results for several different unprocessed meat hydrolysate samples using an MSA hydrolysis procedure. Figure 11 shows a determination of free amino acids in wine. Sample preparation is greatly simplified because IPAD detection is highly sensitive, allowing samples to be diluted 1000-fold or more.

Ordering Information

The *AAA-Direct* is available in three standard configurations for amino acid analysis.

System 1 is the basic configuration. System 2 adds information capability with AS50. In System 3, the AS50 is configured with sample preparation option, as well as sample tray control for sample cooling.

AAA-Certified Gold Electrochemical Cells must be used with the *AAA-Direct* System for amino acid determinations. A Chromeleon® Workstation and the AminoPac PA10 analytical and guard columns are also required and must be ordered separately.

In the U.S., call (800) 346-6390 or contact the Dionex regional office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers.

STANDARD SYSTEM COMPONENTS

Each *AAA-Direct* System includes:

Microbore PEEK GS50 with Vacuum Degas Option ..... P/N 055886

ED50A Detector (includes pH reference electrode) ..... P/N 044094

Microbore Tubing Kit ..... P/N 052324

Application Installation* ..... P/N 038677

Eluent Organizer Set: two EO1 eluent organizers, one Regulator Assembly, and four 2-L plastic bottles ..... P/N 054468

*North America only

SYSTEM 1

***AAA-Direct* Manual System**

Includes BioLC GS50 with degas, BioLC ED50A, LC25 Chromatography Oven, *AAA-Certified* gold amperometry cell for LC25, two EO1 Eluent Organizers, 4 2-L plastic bottles, and the EO1 regulatory accessory. AAA-Direct Installation Kit and 3-day service install are included.

..... P/N 055965

SYSTEM 2

***AAA-Direct* Automated System with Column Heating**

Includes BioLC® GS50 with degas, BioLC ED50A, BioLC AS50 with thermal compartment, *AAA-Certified* gold amperometry cell for AS50, two EO1 Eluent Organizers, 4 2-L plastic bottles, and the EO1 regulatory accessory. Also included are the AutoSelect™ 1.5-mL Vial Cast Tray, and 1.5-mL Vial Kit, (glass with precut septa, 100 each). *AAA-Direct* Installation Kit and 3-day service install are included. ..... P/N 055975

SYSTEM 3

***AAA-Direct* Automated System with Sample Tray Temperature Control and Column Heating**

Includes BioLC GS50 with degas, BioLC ED50A, BioLC AS50 with sample tray temperature control, *AAA-Certified* gold amperometry cell for AS50, two EO1 Eluent Organizers, 4 2-L plastic bottles, and the EO1 regulatory accessory. Also included are the AutoSelect 1.5-mL Vial Cast Tray, and 1.5 mL Vial Kit, (glass with pre-cut septa, 100 each). *AAA-Direct* Installation Kit and 3-day service install are included. ..... P/N 055967

SYSTEM COMPONENT OPTIONS

AminoPac PA10 Analytical Column (2 × 250 mm) ..... P/N 055406

AminoPac PA10 Guard Column (2 × 50 mm) ..... P/N 055407

AAA-Certified Gold ED40 Cell for AS50 ..... P/N 055826

AAA-Certified Gold ED40 Cell for LC25 ..... P/N 055827

AAA-Certified Gold ED40 Cell for LC30 ..... P/N 055828

AAA-Certified Gold Working Electrode ..... P/N 055832

DX LAN Pump Card ..... P/N 044195

Detector Card ..... P/N 044196

Chromeleon/Windows 2000 Small Desktop ..... P/N 060929

Chromeleon/Windows 2000 Mini-Toner Workstation ..... P/N 060931

Chromeleon/Windows 2000 Laptop Bundled Workstation ..... P/N 060931

Specifications

Specifications for the major components are as follows:

COLUMN SPECIFICATIONS

Dimensions:
AminoPac PA10: 2 × 250 mm
AminoPac PA10 Guard Column: 2 × 50 mm

Maximum Operating Pressure:
4000 psi (275 bar)

Mobile Phase Compatibility:
pH 0–14; 0–100% HPLC solvents

Core Particle Substrate Characteristics:
Bead Diameter: 8.5 µm
Pore Size: Microporous, <10 Å
Cross-linking: (% DVB): 55%

Latex Characteristics:
Functional Group: Alkyl quaternary ammonium ion
Latex Cross-linking: 1%
Latex Diameter: 80 nm
Hydrophobicity: Hydrophobic

Capacity:
60 µeq (2 × 250 analytical column)
12 µeq (2 × 50 guard column)

Column Construction:
PEEK with 10-32 threaded ferrule-style end fittings. All components are nonmetallic.

GS50 SPECIFICATIONS

Construction: Chemically inert, metal-free PEEK pump heads and flow paths. Compatible with aqueous eluents of pH 0–14 and reversed-phase eluents.

Type: Dual-piston series pump, microprocessor-controlled constant stroke, variable speed

Control Modes: Remote using TTL or relay, or through Chromeleon chromatography software

Delay volume: Microbore: <600 µL

Maximum Operating Pressure: 5000 psi

Pressure Ripple:
<1% at 0.25 mL/min

Pressure Alarm Limits:
Upper limit 0–35 MPa or 0–5000 psi in one-unit (MPa or psi) increments; lower limit can be set up to one unit lower than upper limit

Flow Rate Range: 0.05–5.00 mL/min

Settable Flow Range:
0.00–5.00 mL/min in 0.01 mL increments

Flow Precision: <0.2 %

Flow Accuracy:
<1% of set value or ± 2 µL/min, whichever is greater

Compressibility Compensation:
User-selectable, based on mobile phase compressibility

Proportioning Type: Low pressure

Compositional Range:
0–100% in 0.1% increments

Compositional Accuracy: ±0.5%

Compositional Precision:
<0.2% at 0.25 mL/min

Gradient Linearity:
Linear and four concave and four convex options

Vacuum Degas: Optional, built-in

Power Requirements: 100–240 V ac, 50/60 Hz (power supply is autosensing, no voltage adjustment required)

Operating Temperature Range:
4–40 °C (40–104 °F); cold room (4 °C) compatible as long as system power remains on

Operating Humidity Range:
5–95% relative, noncondensing

Dimensions (h × w × d): 33.5 × 22.5 × 42 cm (13.1 × 8.8 × 16.4 in.)

Weight: 16.1 kg (35.3 lb); 19.5 kg (43 lb) with degas option

AS50 AUTOSAMPLER SPECIFICATIONS

Sample Capacity:
49 × 10 mL; 100 × 1.5 mL

Vial Size:
10 mL and 1.5 mL pre-cut septum vials

Number of Injections Per Vial:
1–99

Minimum Sample Volume:
1 µL can be sampled from 5 µL in a 100-µL microvial
10 µL can be sampled from a 300-µL microvial
20 µL can be sampled from 500 µL in a 10-mL vial

Variable Volume Range:
1–100 µL in 0.1 µL increments
100 –1000 µL in 1 µL increments

Injection Valve:
PEEK Rheodyne with Tefzel® rotor seal

Injection Loop Size:
25-µL standard; other sizes available

Injection Precision:
Fixed Loop: <0.3% RDS at 20 µL or greater
Variable: >0.5% RSD at 20 µL

Dilution Precision:
≤1.0% RSD for a 1:100 dilution

Dispensing Precision:
<0.2% RSD by weighing; carryover <0.01% with 500 µL flush volume

Ambient Temperature Range:
10–40 °C

Humidity Range:
10–90% relative humidity, noncondensing

Voltage:
90–265 V ac, 47–63 Hz

Power:
300–600 watts with options

Dimensions:
64 × 30 × 46 cm (26 × 12 × 19 in.)

Weight:
<30 kg (<65 lb)

Thermal Compartment Specifications:

Dimensions:
50 × 17.5 × 40 cm (20 × 7 × 16 in.)

Weight:
18 kg (40 lb)

Temperature Control Range:
10–40 °C in 1 °C increments, stable to ±0.2 °C

Ambient Operating Temperature:
20–40 °C

Temperature Stability:
±0.2 °C

Temperature Accuracy:
±0.1 °C

Heatup/Cooldown Time:
5 min from 20–40 °C at ambient 20 °C;
10 min from 40–20 °C at ambient 20 °C

Delay Volume:
Minimal <100 µL

Safety:
Leak sensor and adequate leak handling; liquid spillage drain

Sample Prep Option Specifications:

Dilution Precision:
<1.0% area RSD for a 1:100 dilution (combined dilution and injection)

Dispensing Accuracy:
0.2% by weighing

Sample Tray Temperature Control Specifications:

Sample Vials—Size and Material:
1.5-mL glass and 0.5-mL polypropylene vials

Temperature Control Range:
Programmable from 4–60 °C in 1 °C increments

Temperature Control:
Cooling –20 °C from ambient; heating +40 °C from ambient

Vial Temperature Accuracy:
±2 °C (from 4–10 °C and 41–60 °C); ±1 °C from (11–40 °C)

Vial Temperature Differential:
<2 °C between any two vials

Temperature Stability:
±0.5 °C

Temperature Sensor Accuracy:
±0.25 °C

Temperature Reproducibility:
±0.5 °C

Time to Temperature:
Cools tray from 24 to 4 °C in <30 min; heats tray from 24 to 60 °C in <30 min

ED50A SPECIFICATIONS

Amperometry Electronics:

Noise:
1 pA (dc amperometry);
10 pC (integrated amperometry)

Potential Ranges:
±2.00 V in 0.01-V increments

Control Modes:
Local or remote using relay closures, TTL, or by Chromeleon software through DX-LAN™

Amperometry Flow Cell:

Electrodes:
Interchangeable working electrode—gold, platinum, silver, or glassy carbon
Counter electrode—titanium

Reference Electrode:
pH-Ag/AgCl combination

Wetted Materials:
PEEK, titanium, KEL-F®
polyetherimide, EPR, glass, high density polyethylene (HDPE), and working electrode material (gold, platinum, silver, and glassy carbon)

Cell Volume at Working Electrode:
<0.5 µL

Maximum Operating Pressure:
0.7 MPa (100 psi)

Conductivity Electronics and Flow Cell:
Same as CD20 Conductivity Detector

Output Control:

Filter: 0–10-s response time

Full-Scale Analog Output Ranges
Conductivity: 0.01 µS–3000 µS
Integrated amperometry:
50 pC to 200 µC
dc amperometry: 50 pA to 300 µA

Offset:
To full-scale for each range

Analog Signal Output Voltage:
0.01, 0.1, or 1 V

Autoranging:
Autoranging digital signal monitoring by Chromeleon software through DX-LAN

Physical Specifications:

Power Requirements:
90–265 V ac, 547–63 Hz
(power supply is autosensing—no voltage adjustment required)

Operating Temperature Range:
4–40 °C (40–104 °F);
cold-room (4 °C) compatible as long as system power remains on

Operating Humidity Range:
5–95% relative, noncondensing

Dimensions:
17 × 22.5 × 42 cm
(6.6 × 8.8 × 16.4 in.)

Weight:
8.2 kg (18 lb)

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