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.
**Instrumentation**

- High-performance quaternary gradient microbore pumping system with built-in degasser provides accurate flow, composition control, and low eluent consumption.
- Inert, nonmetallic PEEK (polyetheretherketone) 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. Cumber-some 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.](image1)

![Figure 2. Stability of detector response for 100-pmol standard.](image2)

![Figure 3. Long-term stability of retention time for 1:250 dilution of a cell culture medium sample.](image3)
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 ammonium-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:

- 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 procedure 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 (GaIN) and glucosamine (GlcN), 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.
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.

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.
**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 Chromleon* 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbore PEEK GS50 with Vacuum Degas Option</td>
<td>P/N 055886</td>
</tr>
<tr>
<td>ED50A Detector (includes pH reference electrode)</td>
<td>P/N 044094</td>
</tr>
<tr>
<td>Microbore Tubing Kit</td>
<td>P/N 052324</td>
</tr>
<tr>
<td>Application Installation*</td>
<td>P/N 038677</td>
</tr>
<tr>
<td>Eluent Organizer Set: two EO1 eluent organizers, one Regulator Assembly, and four 2-L plastic bottles</td>
<td>P/N 054468</td>
</tr>
</tbody>
</table>

*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

### Specifications

Specifications for the major components are as follows:

**COLUMN SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Dimensions:</th>
<th>AminoPac PA10: 2 × 250 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AminoPac PA10 Guard Column: 2 × 50 mm</td>
</tr>
<tr>
<td>Maximum Operating Pressure:</td>
<td>4000 psi (275 bar)</td>
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<tr>
<td>Mobile Phase Compatibility:</td>
<td>pH 0–14; 0–100% HPLC solvents</td>
</tr>
<tr>
<td>Core Particle Substrate Characteristics:</td>
<td>Bead Diameter: 8.5 µm</td>
</tr>
<tr>
<td></td>
<td>Pore Size: Microporous, &lt;10 Å</td>
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<tr>
<td></td>
<td>Cross-linking: (% DVB): 55%</td>
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<tr>
<td>Latex Characteristics:</td>
<td>Functional Group: Alkyl quaternary ammonium ion</td>
</tr>
<tr>
<td></td>
<td>Latex Cross-linking: 1%</td>
</tr>
<tr>
<td></td>
<td>Latex Diameter: 80 nm</td>
</tr>
<tr>
<td></td>
<td>Hydrophobicity: Hydrophobic</td>
</tr>
<tr>
<td>Capacity:</td>
<td>60 µeq (2 × 250 analytical column)</td>
</tr>
<tr>
<td></td>
<td>12 µeq (2 × 50 guard column)</td>
</tr>
<tr>
<td>Column Construction:</td>
<td>PEEK with 10-32 threaded ferrule-style end fittings. All components are nonmetallic.</td>
</tr>
</tbody>
</table>

### AminoPac PA10 Analytical Column

- (2 × 250 mm) P/N 055406
- (2 × 50 mm) P/N 055407

### AAA-Certified Gold ED40 Cell

- for AS50 P/N 055826
- for LC25 P/N 055827
- 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 Workstation

- P/N 060929

### Chromeleon/Windows 2000 Mini-Toner Workstation

- P/N 060931

### Chromeleon/Windows 2000 Laptop Bundled Workstation

- P/N 060931
**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

**Sett able 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 x w x 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

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**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-µL 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

**Dispensing Precision:**

- <0.2% RSD by weighing;
- carryover <0.01% with 500 µL flush volume

**Dilution Precision:**

- ±1.0% RSD for a 1:100 dilution

**Dispensing Accuracy:** 1.0% RSD for a 1:100 dilution

**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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