Disposable Electrodes

065040 Revision 08 • April 2016

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Thermo SCIENTIFIC

Gold, (6-pack, polyester), P/N 060139 for Carbohydrates
Gold, (6-pack, PTFE), P/N 066480 for Carbohydrates
Gold, (6-pack), P/N 060082 for AAA-Direct
Silver (6-Pack), P/N 063003
Platinum (6-Pack), P/N 064440
Carbon (6-Pack, PEEK), P/N 069336

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SECTION 1 – INTRODUCTION

1.1. Disposable Electrodes

Disposable electrodes are the latest innovation in electrochemical detection, providing a new level of reproducibility and ease of use. They are less expensive than conventional, non-disposable electrodes and can be replaced more often without electrode reconditioning by polishing and other methods. More frequent replacement of working electrodes results in more predictable and reproducible electrochemical detection. In addition, the use of disposable electrodes can simplify troubleshooting.

1.2. Terminology

Figure 1 shows a gold disposable electrode (DE). When used in conjunction with the 2 mil PTFE Gasket, P/N 060141 (not shown), disposable electrodes are compatible with ED40, ED50 and ED (ICS-3000) electrochemical detectors. The small circle on the DE is the working electrode. The larger circle is a contact pad. The working electrode and contact pad are connected by a straight, narrow lead. All three parts of the DE consist of a thin layer of titanium coating with a top layer of the working electrode material (either Au, Ag, Pt or C). The reverse side (the "dull" side) of the polymer sheet does not carry any metal.

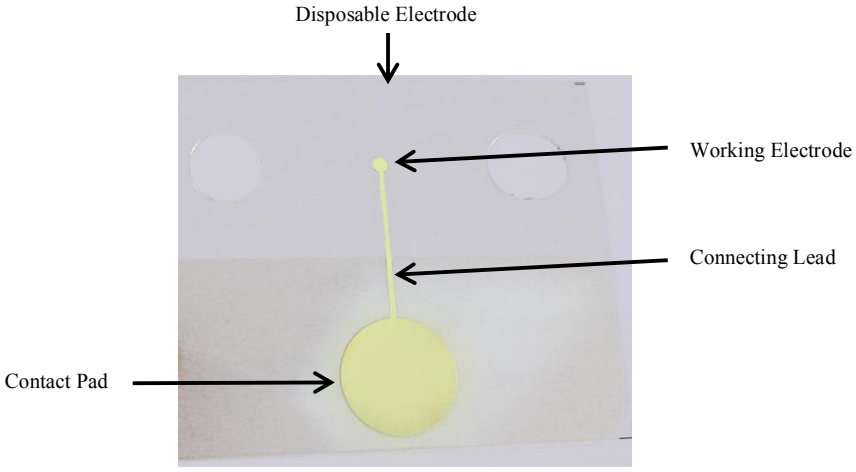


Figure 1 - Disposable Electrode

1.3. Replacement Parts for Electrochemical Cells

Electrochemical cells are used for the detection of a variety of electro-active species, including carbohydrates, amino acids (on a gold working electrode); cyanide, bromide, iodide, thiocyanate, sulfide, thiosulfate (on a silver electrode); alcohols, aldehydes, ketones, carboxylic acids (on a platinum electrode) and catecholamines, S-containing amino acids, electroactive DNA bases and derivatives, etc (on a carbon electrode). The part numbers for the more frequently replaced components of the cell are listed in Table 1.

Table 1 - Replacement Parts

Part Number	Product Description
061756	ED Cell with Reference Electrode and Spacer Block (no working electrode)
062158	ED Cell Polypropylene support block for use with disposable electrodes
061879	ED Cell pH Reference Electrode
060141	Gasket for Disposable Electrode, Pack of 4, ED/ED40/ED50/ED50A Amperometry Cell, .002"
045972	Gasket ED50A/ED50/ED40 Amperometry Cell, 1.0 Mil
060356	ED50A Electrochemical Cell for LC25
060357	ED50A Electrochemical Cell for AS50,TC/CC
060358	ED50A Electrochemical Cell for LC10/20/30
060297	ED50A/50/40/3000 Polypropylene support block for use with disposable electrodes
044198	pH Reference Electrode
048410	O-Ring for the ED40, ED50, or ED50A reference electrode compartment
045939	Blunt 'pogo' to upgrade ED40, ED50, or ED50A cells for compatibility with disposable electrodes
045967	Stop Ring for the ED40, ED50, or ED50A reference electrode compartment

1.4. Waveforms for Disposable Electrodes

Disposable electrodes are manufactured by depositing a thin layer of the working electrode material on a thin polymer film. Care should always be taken when handling these electrodes to ensure that the working electrode is not scratched. Since the working electrode is a very thin deposition, the lifetime of the electrode is compromised by excessively high applied-voltages. Dionex recommends specific waveforms or detection potentials for different applications (see sections 6, 7, 8, 9, 10 and 11 for appropriate waveforms or detection potentials) and only specifies the lifetime of the disposable electrode when used under those conditions.

SECTION 2 – OPERATION AND SYSTEM REQUIREMENTS

2.1. System Requirements

Dionex disposable electrodes are only compatible with Dionex electrochemical detectors, models ED (ICS-3000), ED40 and ED50/ED50A. These electrodes cannot be installed on any other electrochemical detector. Table 2 shows the recommended system components.

Table 2 - System Component Recommendations

ICS-3000 System	Older ICS System
SP/DP Gradient Pump	GS50 or GP50 pump
Manual injector or AS/AS50 autosampler	Manual injector or AS50 autosampler
DC	LC25, LC30 AS50TC column oven
ED	ED40, ED50, or ED50A
Suitable Dionex Column	Suitable Dionex Column

2.2. System Operation Requirements

Dionex systems should be configured to comply with the following key requirements:

1. Mobile phase components kept under helium or nitrogen at all times.
2. On-line degassing of eluents.
3. Accurate and precise flow rates at 0.25 mL/min to 2 mL/min (depending on the application).
4. pH/Ag/AgCl reference electrode.
5. Programmable Integrated Amperometry waveforms with frequencies of 1 Hz or higher.
6. Minimized background signal by contaminants from the system and reagents.
7. Column oven for constant temperature control of the guard column, separation column, and detection cell.

The heat exchange coil in the AS50 thermal compartment must be 0.005” (0.125 mm) inner diameter (ID) PEEK tubing (Dionex P/N 052311) for 2 mm ID column applications.

All tubing between the injector and detector cell inlet must be ≤0.005” (0.125 mm) ID.

SECTION 3 – PURITY REQUIREMENTS FOR CHEMICALS

Obtaining reliable, consistent and accurate results requires eluents that are free from ionic and electrochemically active impurities. Chemicals and deionized (DI) water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also helps protect your ion exchange columns and system components. Dionex cannot guarantee performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

3.1. Deionized (DI) Water

The DI water used to prepare eluents should be Type I reagent grade water with a specific resistance of 18 megohm-cm, or better. The water should be free from ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Ultraviolet (UV) treatment is recommended as part of the water purification. Follow the manufacturer's instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free of electrochemically active surfactants. Expanding their period of use beyond the recommended time may lead to bacterial contamination and as a result, a laborious cleanup may be required. Use of contaminated water for eluents can lead to high background signals and gradient artifacts.

3.2. Sodium Hydroxide

Use 50% w/w sodium hydroxide (Certified Grade, Thermo Fisher Scientific P/N UN 1824) for preparation of all sodium hydroxide eluents.

3.3. Sodium Acetate

Dionex specifies that only sodium acetate purchased from Dionex (P/N 059326) should be used for amino acid analysis by AAA-Direct. Any other source of sodium acetate may contain contaminants that could affect detection. Dionex highly recommends the use of Dionex Sodium Acetate Reagent for carbohydrate analysis; however, anhydrous sodium acetate from Sigma Aldrich (Biochemika Ultra, P/N 71183) is also adequate for inorganic ion analysis. Dionex cannot guarantee proper detection performance when different grades or alternate suppliers of sodium acetate are utilized.

3.4. Methanesulfonic Acid

Use Sigma Aldrich P/N 64280 ≥ 99% methanesulfonic acid (MSA) or equivalent. Dilute as directed.

SECTION 4 – BEFORE YOU START

4.1. The Most Important Rules

4.1.1. Always

a) Use only Dionex recommended reagents for preparation of eluents.
b) Use dedicated glassware and disposable glass or plastic ware for volume adjustments.
c) Keep your eluents blanketed with helium or nitrogen. Prepare new NaOH eluent if left unblanketed for more than 30 minutes.
d) Pull at least 40 mL of new eluent through the lines when changing eluent or adding fresh eluent. This will ensure that your fresh eluent is primed through the lines up to the pump heads.
e) Use proper loop size; oversized sample loops will cause loss of resolution.
f) Use only plastic containers for hydroxide and acetate eluents.
g) Use only glass containers for MSA containing eluents.
h) Transfer MSA with glass pipets.

4.1.2. Never

a) Go to the next step of the installation if the previous step has failed.
b) Start an installation with any of the checklist items below missing.
c) Use 'communal' filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials.
d) Use Methanol or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily. A 20 ppm solution of sodium azide as a rinse fluid may be used as an alternative that does not require daily replacement.
e) Run above 50 ºC or 3,500 psi.

4.2. Initial Checklist

The following items must be available in your lab. The absence of any of these may compromise your analysis.

- ☐ Laboratory water unit delivering 18 megohm-cm water at the installation site.
- ☐ Vacuum pump available for use with the vacuum filtration units.
- ☐ Inert gas cylinder (helium or nitrogen) with a regulator valve (0-200 psi at the low pressure side) and the appropriate size adaptors plus tubing.
- ☐ Performance standard to verify system performance.
- ☐ Sterile-packed 10 mL or 25 mL disposable pipets and suitable pipeting bulbs or pumps.
- ☐ Disposable, plastic (PE) syringe, large-size (at least 20 mL), for priming the pump.
- ☐ Plastic eluent bottles (hydroxide, acetate).
- ☐ Glass eluent bottles (Methanesulfonic Acid, MSA or other acidic eluents).
- ☐ Disposable glass pipets for transferring MSA.
- ☐ Dedicated vacuum filtration unit (Dionex recommends VWR PN 28198-514).

SECTION 5 – INSTALLATION AND START-UP FOR ED (ICS-3000) ELECTROCHEMICAL CELLS

NOTE: This information is also available on the Disposable Electrode Installation Guides shipped with each order. See Appendix B for ED40, ED50, or ED50A cells.

5.1. Comparison of the Conventional Electrode Parts with the Disposable Electrode (DE) Parts

Before proceeding with the installation of disposable electrodes, review the following two figures. These figures will provide a point of reference when installing disposable electrodes.

Figure 2 shows an ED cell body with the reference electrode installed. Figure 3 shows a yoke-knob assembly and spacer block that are standard components of each ED cell. Additionally, Figure 3 also shows the disposable electrode (DE) and the PTFE gasket with a tab. DE is metal-coated on one side only. The reverse side, marked by a label, does not carry any metal.

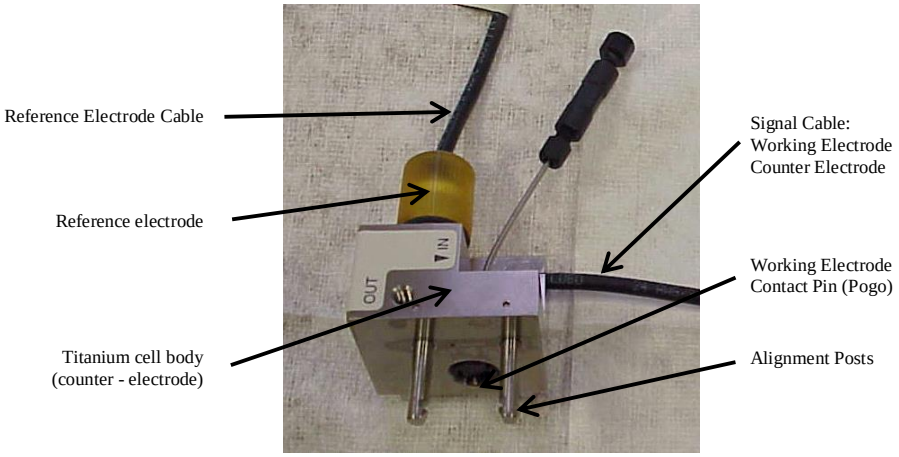


Figure 2 - The Conventional Side
View of the ED (ICS-3000) cell body with reference electrode installed

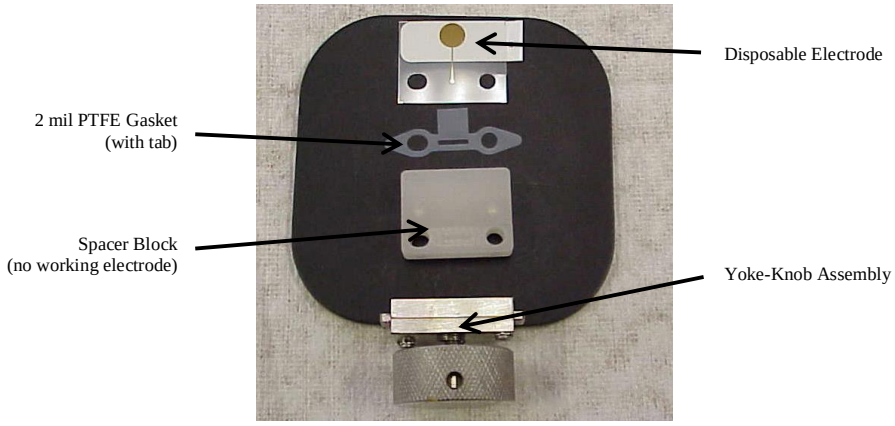


Figure 3 - Parts Required for Disposable Electrode Installation
Spacer block, disposable electrode, and 2 mil PTFE gasket required for disposable electrode usage

5.2. Installation of a Disposable Electrode into a Conventional Detection Cell

When using disposable electrodes with a cell that was originally configured with conventional electrodes, a spacer block must be used instead to properly support the disposable electrodes.
Obtain a spacer block (P/N 062158) prior to installation of the disposable electrode.

WARNING: ***INSTALLING THE DISPOSABLE ELECTRODE OVER THE CONVENTIONAL ELECTRODE IS NOT RECOMMENDED. IT MAY COMPROMISE THE ELECTRODE'S LIFETIME.***

5.3. Installation of a Disposable Electrode into a Cell

Figure 4 shows all parts from Figures 2 and 3 in a single picture. Special attention should be paid to the additional details that are important for the correct use of disposable electrodes.

WARNING: ***WEAR GLOVES WHEN HANDLING THE DISPOSABLE ELECTRODES. FINGERPRINTS CAN CONTAMINATE THE ELECTRODE AND DECREASE PERFORMANCE.***

Prepare a blank spacer block to mount the disposable electrode and Teflon gasket inside the electrode cell.

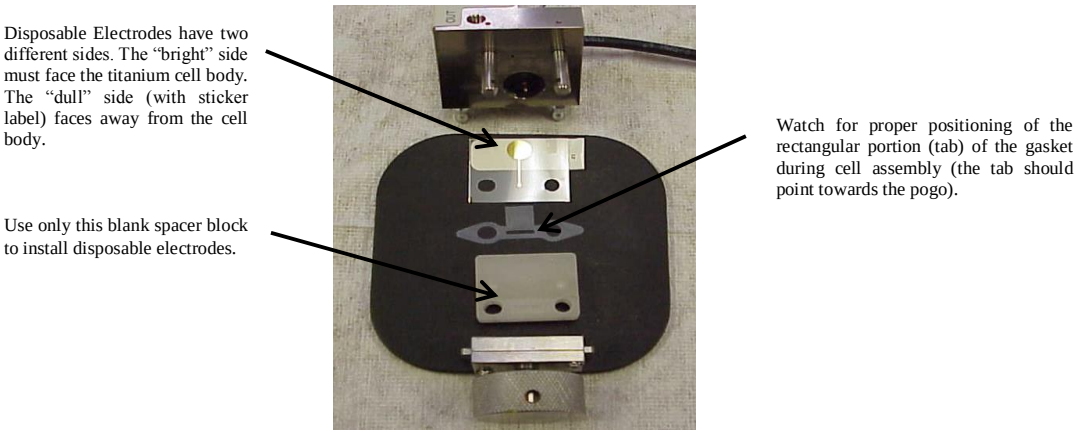


Figure 4 - Overview of Parts Used During Disposable Electrode Installation

NOTE: It is important to properly position the tab. The tab should point toward the pogo chamber.

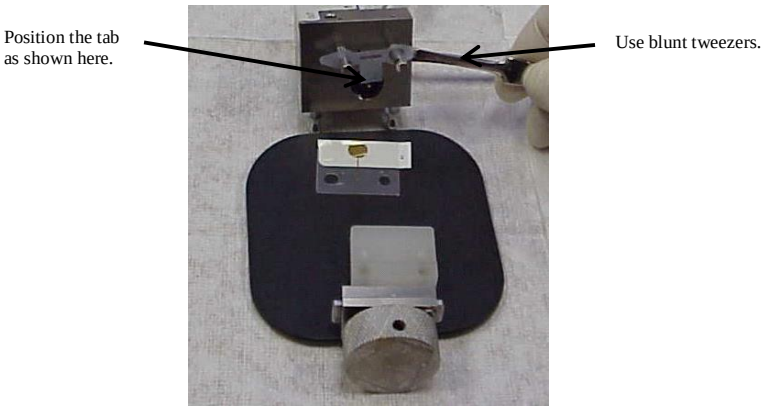


Figure 5 - Installing the gasket for Disposable Electrode

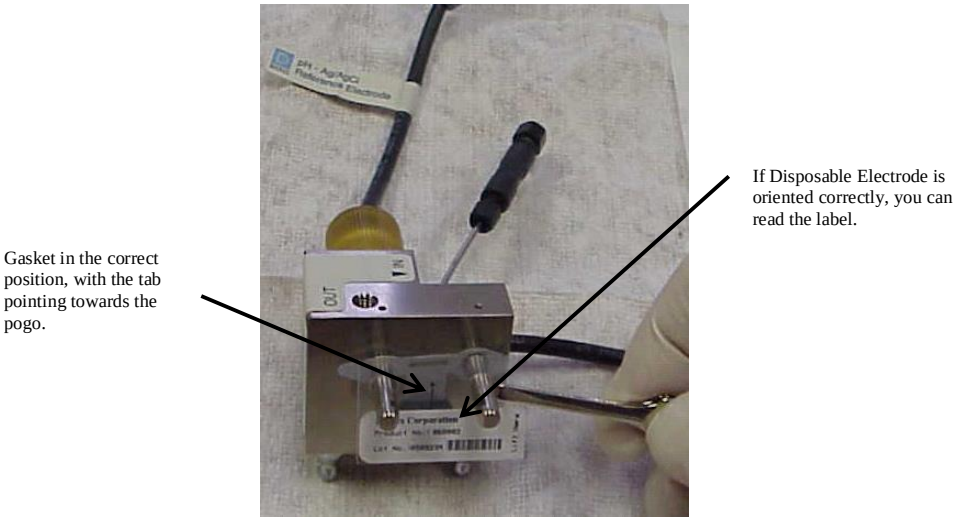


Figure 6 - Installing the Disposable Electrode

The bright side of the DE is the active side and faces the gasket and the cell body. The dull (uncoated) side is recognizable by the label and must face away from the cell body surface.

The working electrode is positioned in the center of the flow path. The flow path is defined by the inner gasket cutout. The tab provides additional sealing for the connecting lead between the working electrode and the circular contact pad.

Figure 7 - Electrode Positioning

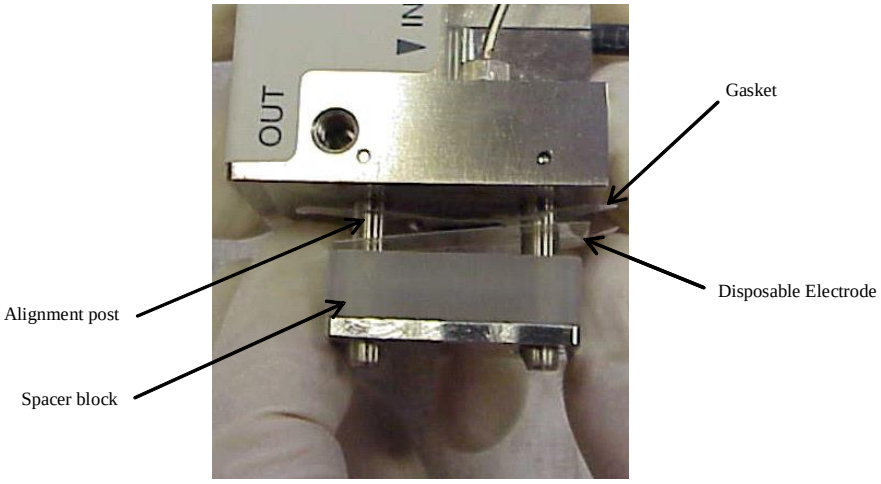


Figure 8 - Placing Spacer Block onto the Alignment Posts

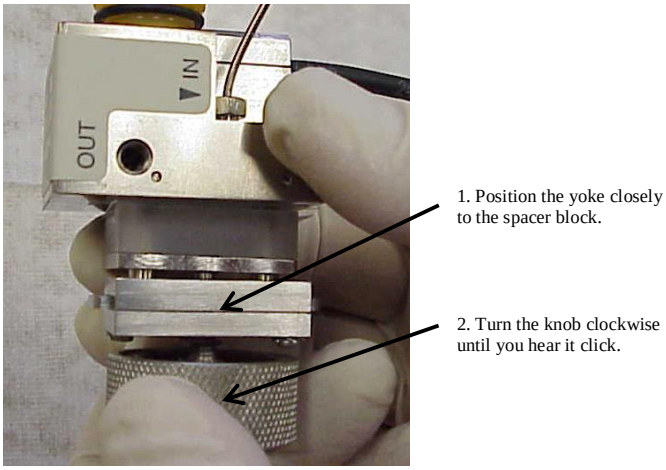


Figure 9 - Final Stage of Cell Assembly: Tightening the Knob

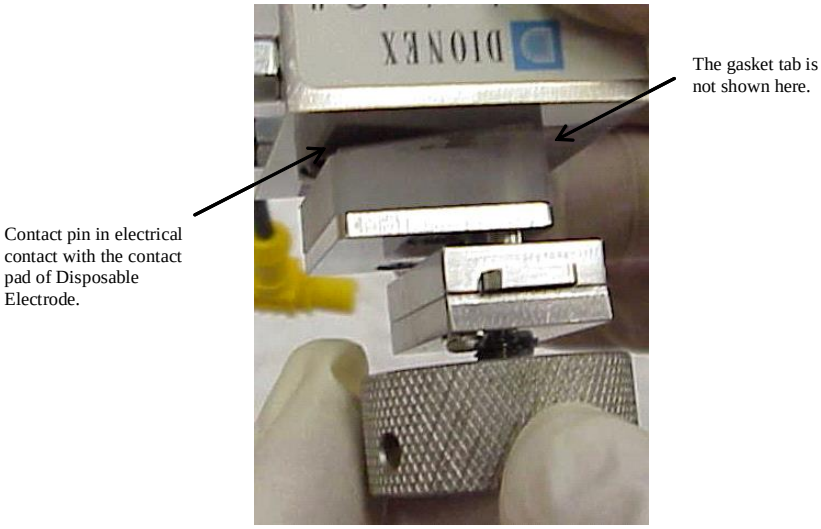


Figure 10 - The Disposable Electrode Installed in the ED (ICS-3000) Cell

SECTION 6 – CARBOHYDRATE ANALYSIS USING A GOLD ELECTRODE

Dionex offers two kinds of disposable gold electrodes for use in carbohydrate analysis. The two kinds differ in the type of polymeric substrates for the working electrode material. The two substrate types are polyester and polytetrafluoroethylene (PTFE).
The following text provides lifetime specifications and recommendation for use with specific examples of applications.

NOTE: ***Always vacuum filter the water through 0.2 µm filters; preferably nylon. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere. On-line degassing is supported through the use of the DP, SP, GP40, GP50, and GS50 gradient pumping systems.***

6.1. Quality Assurance

NOTE: ***Quality Assurance Reports are shipped with each order.***

The specified lifetime of disposable polyester-based gold electrodes for carbohydrate analysis is two weeks. This specification is valid only under the analysis conditions in the QAR for P/N 060139. Specified lifetime for disposable electrodes on PTFE substrate is four weeks. This lifetime specification was developed from testing in 0.75 M NaOH using a flow rate of 0.4 mL/min with detection cell at 25 ºC. Lifetime specifications for disposable electrodes have been developed from long term experiments under carefully controlled conditions. Actual lifetimes may vary depending on additional parameters specific to different applications.

6.2. Preparation of Carbohydrate Standard

The Dionex MonoStandard, Mix of Six, (P/N 043162) contains 100 nmol each of L-Fucose, D-Galactosamine, D-Glucosamine, D-Galactose, D-Glucose, and D-Mannose. Follow these instructions:

a. Reconstitute the standard by adding 1.0 mL DI water to the vial containing the dry monosaccharide standard. The concentration of each of the monosaccharides will then be 100 µM.
b. Take a 100 µL aliquot of the diluted standard and add 900 µL to make a 10 µM solution.
c. Inject 10 µL volumes to compare with the electrode test chromatogram, when installing a new electrode or column, or when troubleshooting a system.

6.3. Recommended Waveforms

Carbohydrate oxidation at gold electrodes is made possible by a rapid sequence of potentials (waveform) adjusted between the working electrode (gold) and the reference electrode (Ag/AgCl). Resulting currents are measured by integration during a short time interval of the detection waveform. The standard carbohydrate waveform recommended for both types of gold disposable electrodes (PTFE and Polyester) is shown in Table 3.

Table 3 - Quadruple Potential (QP) Waveform for Carbohydrate Analysis

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl	Integration
0.00	+0.10	
0.20	+0.10	Begin
0.40	+0.10	End
0.41	-2.00	
0.42	-2.00	
0.43	+0.60	
0.44	-0.1	
0.50	-0.1	

WARNING: ***NEVER POLISH disposable gold electrodes. This will damage the electrode.***

6.4. Part Numbers

The different packages of gold disposable working electrodes available are listed below. Part numbers for additional ED parts are listed in Section 1.3. Suitable Dionex columns for each application are specified in the respective figures throughout this document.

ED (ICS-3000), ED40, ED50, or ED50A

060139 Carbohydrate Disposable gold electrodes (polyester), pack of 6 electrodes and six 0.002" gaskets.
060216 Carbohydrate Disposable gold electrodes (polyester), 24 electrodes (4 bundled packages of P/N 060139).
066480 Carbohydrate Disposable gold electrodes (PTFE), 6 electrodes and six 0.002” gaskets.

6.5. Applications

6.5.1 Common Monosaccharides from Mammalian Glycoproteins

Many proteins have carbohydrates attached to them. The presence of a carbohydrate can control the biological activity of the protein or the rate at which it is cleared from the system. For example, certain glycosylated forms of tissue plasminogen activator (tPA) have more enzymatic activity than others. Erythropoeitin shows complex effects if the protein is deglycosylated or the glycosylation is altered. Failure to be secreted from the body, decreased stability, and decreased biological activity occurs if multiple glycosylation sites are eliminated. Desialylation and/or less branched oligosaccharides give increased activity *in vitro*, but decreased activity *in vivo*. Thus protein glycosylation is important to many scientists, including those making recombinant proteins for therapeutic use. The following conditions should be used for guidance only. These conditions may be modified, as necessary, to suit your particular application needs.

Column:	CarboPac PA20 and Guard	
Gradient:	12 mM NaOH, 200 mM NaOH Regeneration for 6 min	**Peaks**
Flow Rate:	0.5 mL/min	1. Galactosamine
Detection:	Pulsed Electrochemical Detection, Au electrode *	2. Glucosamine
Waveform:	Quadruple Potential	3. Galactose
Sample:	30 µL Reconstituted hydrolyzed serum	4. Glucose
		5. Mannose

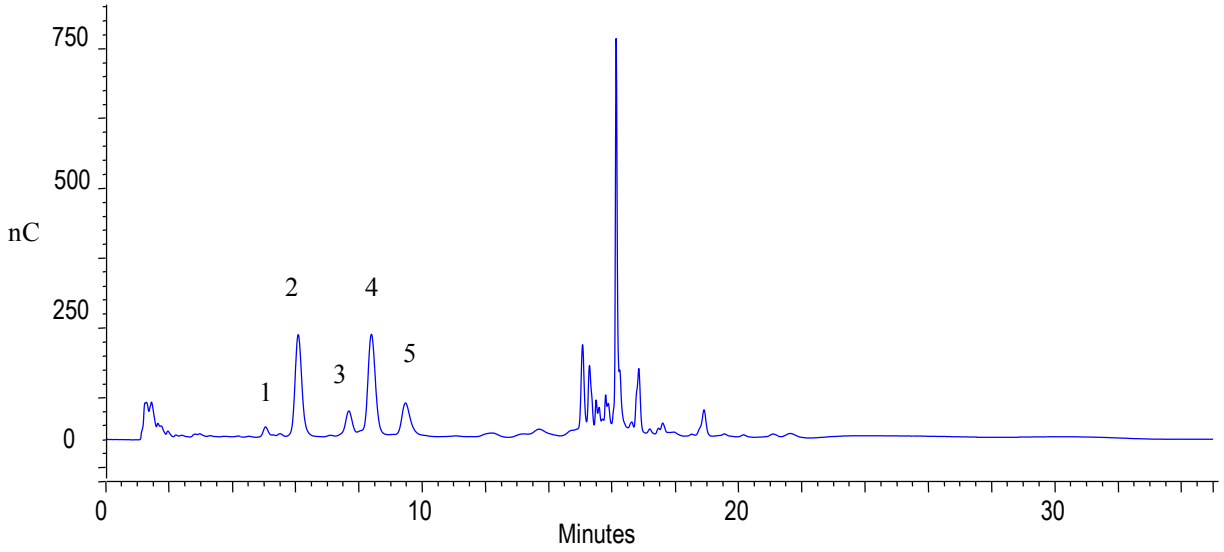


Figure 11 - Analysis of Monosaccharides from Hydrolyzed Rabbit Serum

Sample Preparation: 280 µL of 50% rabbit serum was hydrolyzed in 4 M TFA for 4 hours at 100 °C. The sample was vacuum-dried, reconstituted in 560 µL of water, and then filtered through a 0.45 µm nylon filter.

* Polyester or PTFE-based disposable electrodes are recommended for this application.

6.5.2. Profiling of Inulins

Inulins and fructo-oligosaccharides (FOS) are increasingly being used as functional food ingredients. Chain length distribution profiles of commercial products such, as those derived from inulin, can be determined by using HPAE-PAD with gradient elution. By adjusting the initial gradient profile, smaller oligofructose chains can be distinguished from the inulin chains and separations exceeding the degree of polymerization (DP) 80 are possible.

Quantification of individual inulin oligomers requires knowledge of the PAD response factors. These have been determined for the Fn and GFn oligomers from DP 2 to 8 and from DP 11 to 17, by isolation of 5-20 mg quantities of the pure oligomers using preparative scale RP, HPLC. Unfortunately, pure fractions in the range DP 6-10 could not be obtained due to coelution problems. For DP > 17, the response factor appears to change very slowly with increasing DP, and relative response factors can be obtained by interpolation. Semi-preparative scale (9-mm and 22-mm ID) CarboPac PA1, PA100, and PA200 columns are available and can be used for isolation of milligram quantities of all inulin oligomers.

Columns:	CarboPac PA200 (3 x 250 mm) or CarboPac PA100 (4 x 250 mm)
Gradient:	120-320 mM sodium acetate in 100 mM NaOH over 40 min
Flow Rate:	PA200: 0.5 mL/min
	PA100: 1.0 mL/min
Detection:	Pulsed amperometry, quadruple potential waveform, gold electrode *
Sample:	Inulin from chicory (Sigma)

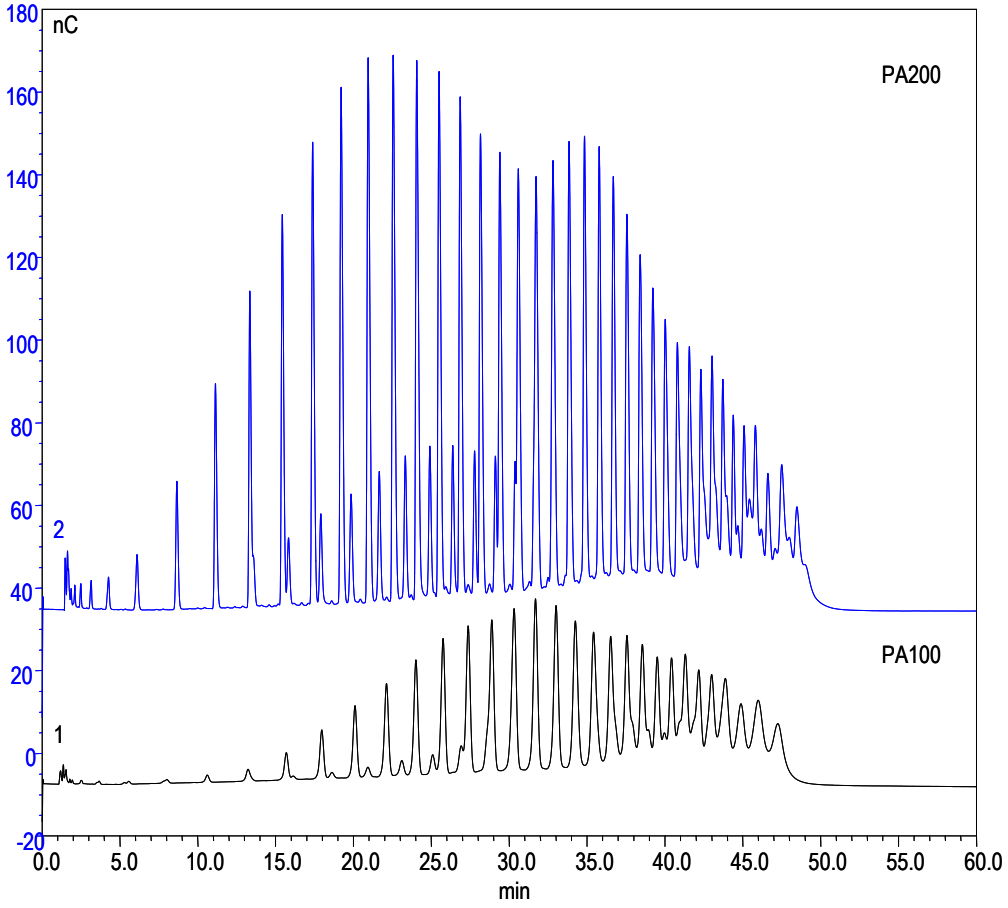


Figure 12 - Profiling of Inulin with Two Different Columns

* PTFE-based disposable electrode is recommended for this application.

SECTION 7 – AMINO ACIDS ANALYSIS USING A GOLD WORKING ELECTRODE

NOTE: PTFE-based disposable electrodes are not recommended for amino acid analysis.

7.1. Quality Assurance

NOTE: Quality Assurance Reports are shipped with each order.

Specified lifetime of disposable gold electrodes (polyester) for amino acid analysis is one week. This specification is valid only for the waveform shown in the QAR for P/N 060082 and with gradient elution methods presented in the Dionex AAA Direct manual. Lifetime specifications for disposable electrodes have been developed from long term experiments under carefully controlled conditions. Actual lifetimes may vary depending on additional parameters not included in the long term experiments carried out at Dionex.

7.2. Testing with Histidine Standard

a) Make an 8 µM solution of histidine by adding 1.0 mL of DI water to the dry residue in the micro vial shipped as a PN 059568 (Histidine Standard, AAA-Direct Installation). Vortex and shake for one minute.
b) Alternatively, prepare your own 8 µM histidine standard by first preparing an 8 mM solution of histidine in 0.1 M HCl. In the second step, prepare the 8 µM standard by a 1000-fold dilution with a 20 ppm solution of sodium azide.
c) Install an AminoPac PA10 column and adjust the eluent composition and flow rate (175 mM sodium hydroxide, 25 mM sodium acetate and 0.25 mL/min). With the disposable gold electrode (AAA, polyester) installed in the detection cell apply the waveform of Table 4 (Section 7.3). Select 30ºC and 25ºC for the column and detection cell respectively. Inject 25 µL of the 8 µM standard. The peak height, background and noise should be as specified in the Quality Assurance Report shipped with each six pack of AAA Disposable Electrodes.
d) If this is not the case, see the Section 12 of this manual or the Troubleshooting Section of the AminoPac PA10 manual.

7.3. Recommended Waveforms

Table 4 - Waveform for Integrated Amperometric Detection of Amino Acids with Gold Electrodes

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl)	Potential (V) vs. pH	Integration
0.000	-0.20	+0.13	
0.040	-0.20	+0.13	
0.050	0.00	+0.33	
0.210	0.00	+0.33	Begin
0.220	+0.22	+0.55	
0.460	+0.22	+0.55	
0.470	0.00	+0.33	
0.560	0.00	+0.33	End
0.570	-2.00	-1.67	
0.580	-2.00	-1.67	
0.590	+0.60	+0.93	
0.600	-0.20	+0.13	

WARNING: ***NEVER POLISH or TOUCH disposable gold electrode surface. This will damage the disposable electrode.***

7.4. Part Numbers

The following part numbers refer to AAA-Direct certified gold disposable electrodes and conventional gold electrode replacement. Additional part numbers are listed in Section 1.3. Suitable Dionex columns are specified in the figures throughout the manual.

ED (ICS-3000), ED40, ED50, or ED50A

060082 AAA-Direct Disposable Gold Working Electrodes (polyester), Pack of 6 and six .002" gaskets.

060140 AAA-Direct Disposable Gold Working Electrodes (polyester), 4 Bundled Packages of 6 and twenty four .002" gaskets.

ED (ICS-3000)

063722 ED Conventional Working Electrode, AAA-Direct, with three .001” gaskets and polishing kit.

ED40, ED50, or ED50A

055832 ED50A , with gasket and polishing kit.

7.5. Example Applications

For more example separations, please refer to the AminoPac PA10 manual for AAA-Direct. The AminoPac PA10 manual also contains all the method details for the separation listed below.

7.5.1. MSA Hydrolysis of Meat Samples

Samples were hydrolyzed using 4 M methanesulfonic acid. As shown in Figure 13, samples hydrolyzed by that technique may contain carbohydrates and the use of the Gradient Conditions from Table 5, "Gradient Conditions for Amino Acids and Carbohydrates," is thus recommended. Note that the two amino sugars also appearing in the chromatograms are separated by both gradient methods from Table 5 or Table 6, "Gradient Conditions for Protein Hydrolysates"; therefore, the method in Table 5 is recommended for meats or other foods with high sugar content.

NOTE: The Table 6 gradient conditions will cause glucose and alanine to co-elute.

Sample Preparation:	Hydrolyze 0.1 g of meat in 5.0 mL of 4.0 M MSA for 16 hours at 100 °C. Dilute 5x with water. In the next dilution step, dilute 500 fold with 8.0 µM norleucine diluent.
Injection Volume:	25 µL
Sample Concentration:	8.0 µM, all amino acids
Column:	AminoPac PA10 analytical and guard columns
Column temperature:	30 °C
Expected System Operating Backpressure:	< 3,000 psi
Eluent:	
E1:	Deionized water
E2:	250 mM NaOH
E3:	1 M Sodium acetate
Eluent Flow Rate:	0.25 mL/min
ED50 waveform:	See Table 4
Gradient Conditions:	See Table 5

1. Arginine
2. Hydroxylsine
3. Lysine
4. Galactosamine
5. Glucosamine
6. Glucose
7. Alanine
8. Threonine
9. Glycine
10. Valine
11. Hydroxyproline
12. Serine
13. Proline
14. Isoleucine
15. Leucine
16. Methionine
17. Norleucine
18. Histidine
19. Phenylalanine
20. Glutamate
21. Aspartate
22. Cystine
23. Tyrosine

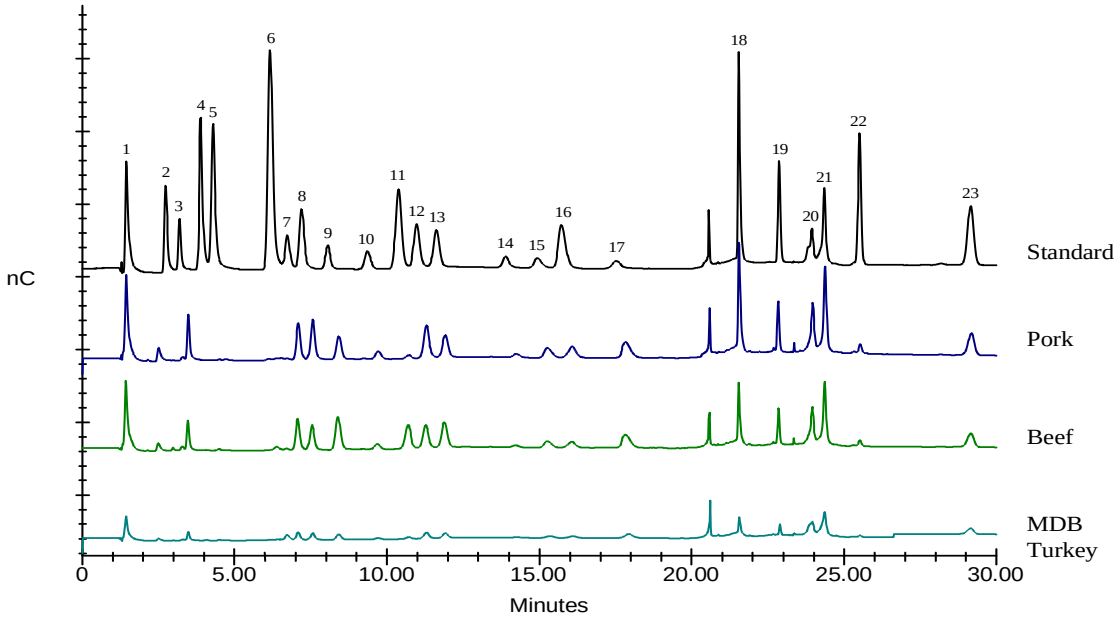


Figure 13 - Analysis of Meat Hydrolysates

Table 5 - Gradient Conditions for Amino Acids and Carbohydrates (Flow 0.25 mL/min)

Time (min)	%E1	%E2	%E3	Curve	Comments
Init	84	16	0		Autosampler fills the sample loop
0.0	84	16	0		Valve from Load to Inject
2.0	84	16	0		Begin hydroxide gradient
12.1	68	32	0	8	
16.0	68	32	0		Begin acetate gradient
24.0	36	24	40	8	
40.0	36	24	40		
40.1	20	80	0	5	Column wash with hydroxide
42.1	20	80	0		
42.2	84	16	0	5	Equilibrate to starting conditions
65.0	84	16	0		

Table 6 - Gradient Conditions for Protein Hydrolysates (Flow 0.25 mL/min)

Time (min)	%E1	%E2	%E3	Curve	Comments
Init	76	24	0		Autosampler fills the sample loop
0.0	76	24	0		Valve from Load to Inject
2.0	76	24	0		Begin hydroxide gradient, valve back to Load
8.0	64	36	0	8	
11.0	64	36	0		Begin acetate gradient
18.0	40	20	40	8	
21.0	44	16	40	5	
23.0	14	16	70	8	
42.0	14	16	70		
42.1	20	80	0	5	Column wash with hydroxide
44.1	20	80	0		
44.2	76	24	0	5	Equilibrate to starting conditions
75.0	76	24	0		

7.5.2. Simultaneous Monitoring of Amino Acids and Carbohydrates in Fermentation Broths

Dionex recommends the use of a special gradient for the separation of amino acids typically found in fermentation broth samples. The gradient modification, see Table 5, is necessary in order to separate the glucose and alanine peaks. These two peaks co-elute using the conditions in Table 6. Use the same waveform as listed in Table 4.

Injection Volume:	25 µL of broth after filtration (0.4 µm filter) and 1000x dilution with DI water.
Column:	AminoPac PA10 analytical and guard columns
Column temperature:	30 °C
Expected System Operating Backpressure:	< 3,000 psi
Eluent:	
E1:	Deionized water
E2:	250 mM NaOH
E3:	1 M Sodium acetate
Eluent Flow Rate:	0.25 mL/min
ED50 waveform:	See Table 4
Gradient Conditions:	See Table 5

1. Arginine
2. Lysine
3. Glutamine
4. Glucose
5. Alanine
6. Threonine
7. Glycine
8. Valine
9. Serine
10. Proline
11. Isoleucine
12. Leucine
13. Methionine
14. Histidine
15. Phenylalanine
16. Glutamate
17. Aspartate
18. Cystine
19. Tyrosine

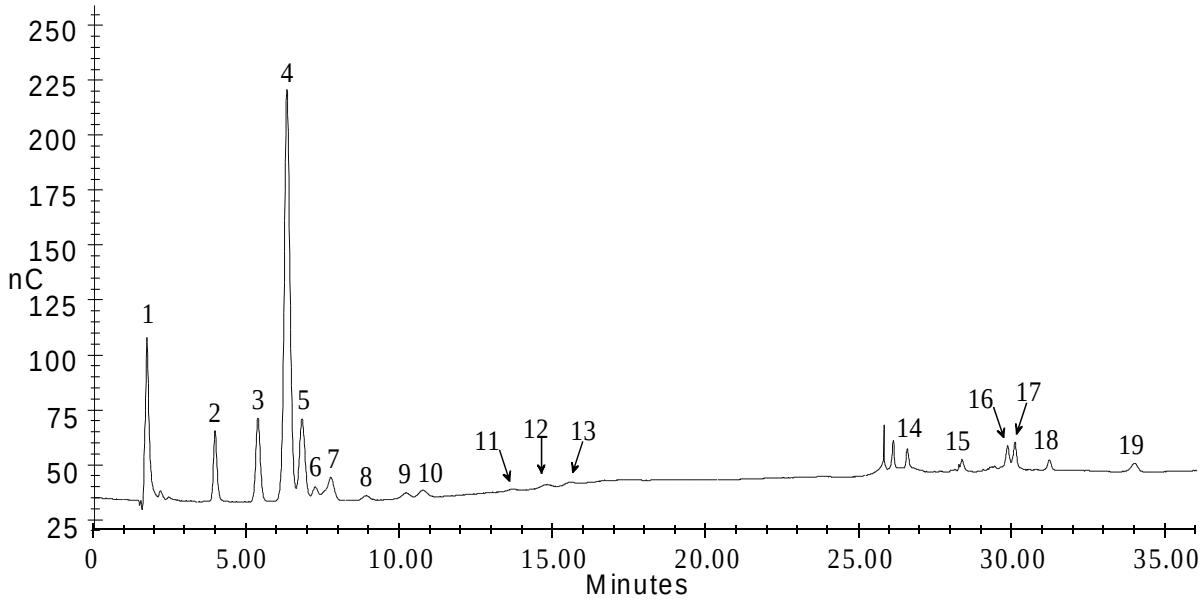


Figure 14 - Simultaneous Monitoring of Amino Acids and Glucose in Fermentation Broths

SECTION 8 – CYANIDE, SULFIDE, BROMIDE, IODIDE, THIOCYANATE AND THIOSULFATE USING SILVER ELECTRODE

NOTE: ***Always vacuum filter the water through 0.2 µm filters, preferably nylon. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere. On-line degassing is supported through the use of the DP, SP, GP40, GP50 and GS50 gradient pumping systems and the IS20 and IS25 isocratic pumping systems.***

8.1. Quality Assurance

NOTE: ***Quality Assurance Reports are shipped with each order.***

The specified lifetime of disposable silver electrodes is two weeks. This specification is only valid under the analysis conditions in QARs for P/N 063003. Lifetime specifications for disposable electrodes have been developed from long term experiments under carefully controlled conditions. Actual lifetimes may vary depending on additional parameters not included in the long term experiments carried out at Dionex.

8.2. Recommended Waveforms

There are several waveforms that can be used for the analysis of cyanide, sulfide, bromide and thiosulfate. The choice of waveform depends upon the concentration of sulfide in the sample, and the goal of the analysis.

The waveform in Table 7 is the best choice when comparatively low concentrations of sulfide are present, as long as the sulfide concentration is not more than about 10 ppm. The waveform in Table 8 is recommended for high concentrations of sulfide, but produces more noise. The waveform in Table 9 is recommended when the analysis of bromide or thiocyanate in the absence of sulfide is the goal.

Table 7 - Waveform for Silver Electrodes and Low Sulfide

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl	Integration
0.00	-0.10	
0.20	-0.10	Start
0.90	-0.10	End
0.91	-1.00	
0.93	-0.30	
1.00	-0.30	

Table 8 - Waveform for Silver Electrodes and High Sulfide

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl	Integration
0.00	-0.10	
0.20	-0.10	Start
0.90	-0.10	End
0.91	-1.15	
0.93	-0.30	
1.00	-0.30	

Table 9 - Waveform for Bromide and Thiocyanate Detection

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl	Integration
0.00	0.05	
0.20	0.05	Start
0.90	0.05	End
0.91	-0.40	
1.00	-0.40	

8.3. Part Numbers

The following part numbers refer to disposable silver and conventional silver electrodes. Additional part numbers are listed in Section 1.3. Suitable Dionex columns are specified in the figures throughout this document.

ED (ICS-3000)
061755 ED Conventional Working Electrode, Ag, with three .001" gaskets and polishing kit.

ED40, ED50, or ED50A
044114 Conventional Silver Working Electrode, with gasket and polishing kit.

ED (ICS-3000), ED40, ED50, or ED50A
063003 Silver Disposable Electrodes, Pack of 6 and six 0.002" gaskets.

8.4. Applications

For a more comprehensive showing of example separations, please refer to the column manual for your specific column. The column manual also contains the method details for the separations listed below.

8.4.1. Simultaneous Determination of Cyanide and Sulfide

Liquid chromatography (LC) with direct current (DC) amperometry is a sensitive method for the separation and detection of sulfide and cyanide. However, when sulfide and cyanide are detected in real samples by DC amperometry on a silver working electrode, the electrode surface is frequently fouled. Although a fouled electrode can be reconditioned, the process is cumbersome and time consuming. Recently, a method was published, using a disposable silver electrode, showing that the application of a pulsed waveform is an effective method of preventing electrode fouling. The details of the method are described in the following references: J. Cheng, P. Jandik, N. Avdalovic, *Anal. Chim. Acta* 536 (2005) 267-274, and also in the Ion Pac AS7 column manual. The chromatogram below shows an improved separation of cyanide and sulfide using an updated method and a disposable silver electrode.

8.4.1.1. Preparation of Cyanide Standard Solution

a. Weigh 0.1885 g sodium cyanide and dissolve with 10g of 0.25 M sodium hydroxide to obtain a 1% cyanide (10,000 ppm) solution.
b. Dilute to the concentration you need (between 30 to 3,000 ppb) with the 0.25 M sodium hydroxide solution, e.g. weigh 0.10 g of 10,000 ppm cyanide solution, dilute it 100 times with 0.25 M sodium hydroxide to obtain 100 ppm, and then repeat the procedure for further dilution to 1 ppm cyanide.

Injection Volume:	25 µ	L	Peaks:
Sample Conc.:	See chromatogram		1. Cyanide
Column:	IonPac AS7 (2 x 250 mm)		2. Sulfide
	Guard (2 x 50 mm)		
Eluent:	100 mM sodium hydroxide, 200 mM sodium acetate		
	7.5 mM ethylenediamine (EDA)		
Flow Rate:	0.25 mL/min		
Detection:	Integrated amperometry,		
	Disposable silver electrode, Waveform of Table 7		

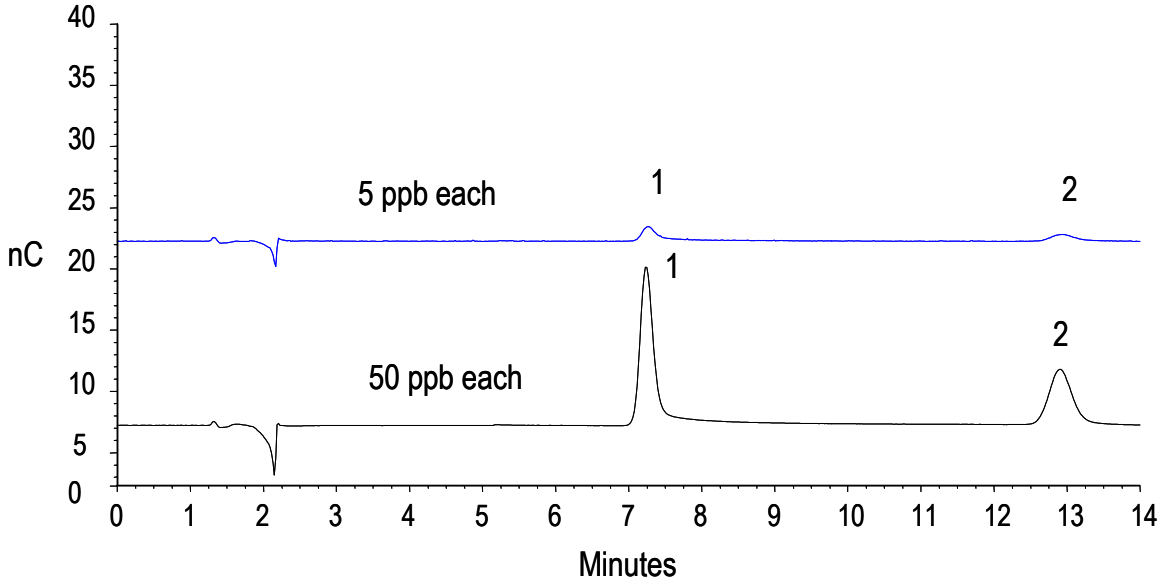


Figure 15 - Detection of Cyanide and Sulfide

8.4.1.2. System Suitability Testing with Cyanide Standard

The suitability of a system for low level cyanide analysis can be confirmed/restored by the following procedure:

a. Install IonPac AS7 column and IonPac AG7 Guard (both ID 2mm). Select 30ºC for column temperature.
b. Install a new disposable silver electrode. Select 25ºC for the detection compartment if working with ICS-3000. Otherwise, maintain the cell at the same temperature as the column.
c. Prepare an eluent containing 75 mM sodium hydroxide, 250 mM sodium acetate and 7.5 mM ethylenediamine. Adjust the flow rate at 0.25 mL/min.

   Note: EDA is used for preventing interference by transition metals with the detection of cyanide on silver electrodes. The EDA concentration should be kept at the optimal value 7.5 mM. But the concentrations of sodium hydroxide and sodium acetate can be adjusted for achieving satisfied separation

d. Apply the waveform from Table 7 of the Product Manual for Disposable Electrodes. An injection of a 50 ppb standard of cyanide should result in a well defined peak with a peak area >1.0 nC min and with asymmetry in the range of 0.9 to 2.0.
e. If there is a discernible peak but with a peak area <1.0 nC min and/or asymmetry value >2.0, the performance is usually improved by pumping the EDA-containing eluent at 0.25 mL/min for about 1-2 hours. If the rinse with the EDA-containing eluent did not improve the peak parameters (or if there was no discernible peak), rinse the column with 2 M nitric acid for approximately 30 min at 0.25 mL/min.

   IMPORTANT: Disconnect the detection cell from the column for the nitric acid rinse. Rinse with water to replace the eluent before the nitric acid rinse and repeat the water rinse after the nitric acid rinse until the pH becomes neutral. In the next step, condition the column with at least 5 mL of the eluent, install a new disposable electrode and repeat the injection of 50 ppb cyanide standard. The detection performance should be restored at this point.

f. An asymmetry value >2.0 indicates a need for replacement of either the guard column or the analytical column. Carry out an injection of 50 ppb cyanide standard without the guard column to determine the status of the analytical column.

8.4.2. Detection of Iodide

NOTE: ***There are two waveforms that can be used for the analysis of iodide, thiosulfate and thiocyante. The choice of waveform depends upon which analytes are present in the sample, and the goal of the analysis. The waveform in Table 7 is a good choice for samples that do not contain thiocyanate, or if thiocyante detection is not the goal. The waveform in Table 9 is recommended when the analysis of thiocyanate is the objective.***

8.4.2.1. Preparation of Iodide Standard Solution

a. Weigh 0.1181 g sodium iodide.
b. Dissolve the whole amount with 10 g of DI water to obtain a 1% iodide (10,000 ppm) solution.
c. Dilute to a lower concentration (between 30 to 3,000 ppb) with water, e.g. take 0.10 g of 10,000 ppm iodide, dilute 100 times with water to obtain a 100 ppm solution of iodide, and then repeat the procedure to further dilute to a 1 ppm iodide solution.

NOTE: ***All standards should be stored in a refrigerator.***

8.4.2.2. Detection of Iodide and Thiocyanate

The example below shows the simultaneous analysis of iodide and thiocyante in a single run, using the waveform shown in Table 9. Notice that the Quality Assurance Report uses the waveform of Table 7, since there is no thiocyanate in that sample.

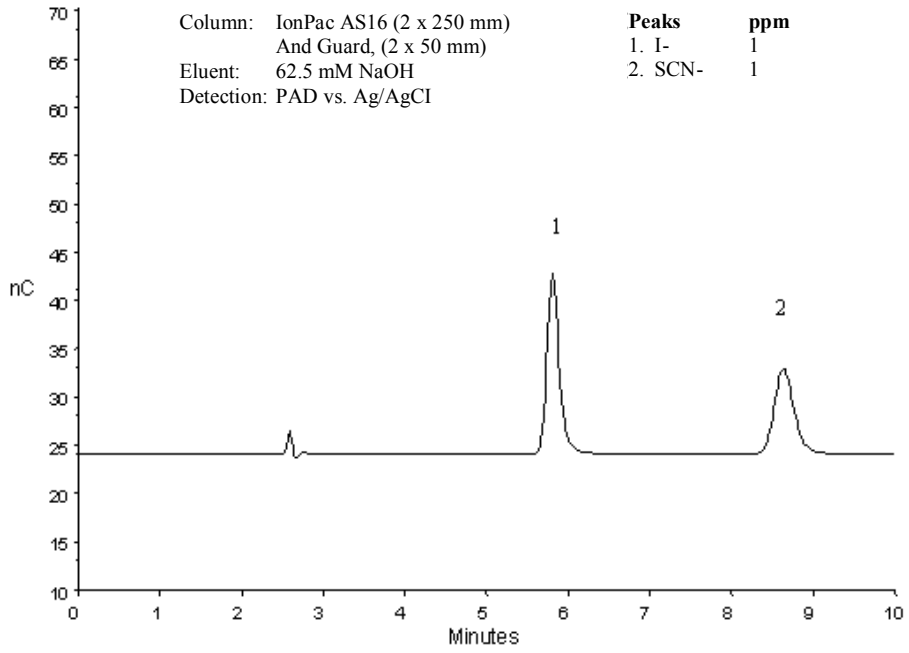


Figure 16 - Separation of Iodide and Thiocyante

SECTION 9 – ALCOHOLS AND CHELATING AGENTS USING A PLATINUM ELECTRODE

NOTE: Use only glass containers for MSA containing eluents.

9.1. Quality Assurance

NOTE: Quality Assurance Reports are shipped with each order.

Specified lifetime of disposable platinum electrodes for alcohol analysis is two weeks. This specification is valid only under the analysis conditions in QARs for P/N 064440. Lifetime specifications for disposable electrodes have been developed from long term experiments under carefully controlled conditions. Actual lifetimes may vary depending on additional parameters not included in the long term experiments carried out at Dionex.

9.2. Recommended Waveforms

Previously published waveforms include current integration periods at intermediate potentials such as 0.30 V vs. Ag/AgCl. As shown by Dionex authors, integration at the highest potential of a waveform improves overall performance of platinum electrodes. The details of the method are described in the following reference:
J. Cheng, P. Jandik, X. Liu and C. Pohl, J. Electroanal. Chem. 608(2007), 117-124.

Table 10 - Pulsed Amperometric Detection (IPAD) Waveform for Platinum Electrodes

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl	Integration
0.00	0.30	
0.31	0.30	
0.32	1.15	
0.64	1.15	Start
0.66	1.15	End
0.67	-0.30	
1.06	-0.30	
1.07	-0.30	

9.3. Part Numbers

The following part numbers refer to disposable conventional platinum working electrodes. Additional part numbers are listed in Section 1.3. Suitable Dionex columns are specified in the figures throughout this document.

ED (ICS-3000), ED40, ED50, and ED50A
064440 Platinum Disposable Electrodes, Pack of 6 and six 0.002" gaskets.
043700 Knitted Reaction Coil 375 µL.

ED (ICS-3000)
061751 ED Conventional Working Electrode, Pt, with three .001” gaskets and polishing kit.

ED40, ED50, and ED50A
044113 Conventional Platinum Working Electrode with gasket and polishing kit

9.4. Applications

For a more comprehensive showing of example separations, please refer to the manual for your specific column. The column manual also contains all the method details for the separations listed below.

9.4.1. Separation of Alcohols

The analysis of alcohols in a number of different matrices is important for a variety of reasons. One very important analysis is the determination of alcohols in a cell culture medium. Ion exclusion chromatography and integrated amperometric detection provide a direct, reliable and sensitive method for the determination of alcohols. The example below shows the simultaneous analysis, alcohols, glycols and glycerol.

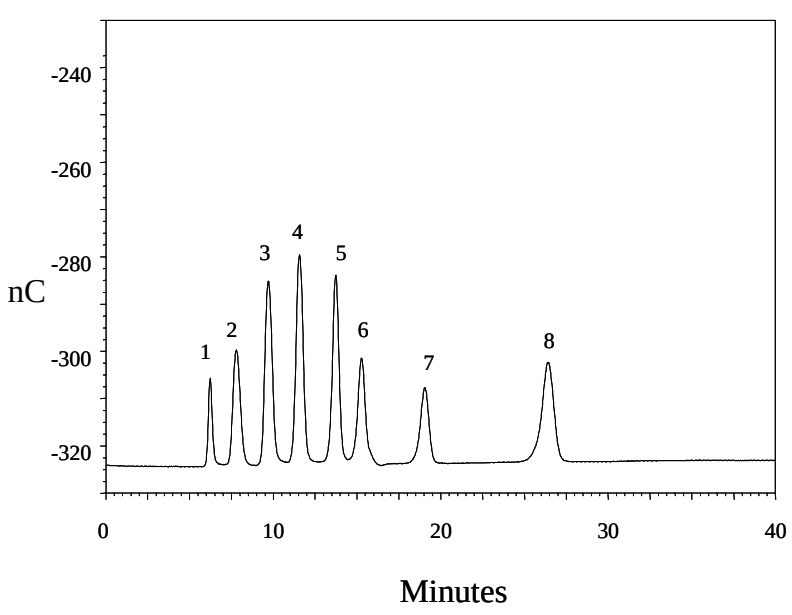
9.4.1.1. Preparation of Standards and Eluents

9.4.1.1.1. Preparation of Glycerol Standard

a. Weigh in 0.20 g glycerol into a scintillation vial.
b. Dissolve the whole amount with 19.80 g of pure water to obtain a 1% glycerol (10,000 ppm) solution.
c. Take 0.10 g of 10,000 ppm glycerol, dilute 200 times with water to obtain a 50 ppm solution of glycerol.

9.4.1.1.2. Preparation of 0.10 M Methanesulfonic Acid (MSA)

a. Filter ~990 mL water through a 0.2 µm nylon filter into a glass eluent container (1.0 L).
b. Using a glass Pipette, pipette 6.6 mL (9.71g) of 99% MSA into the 1.0 L glass container.
c. Fill up to 1.0 L with water.



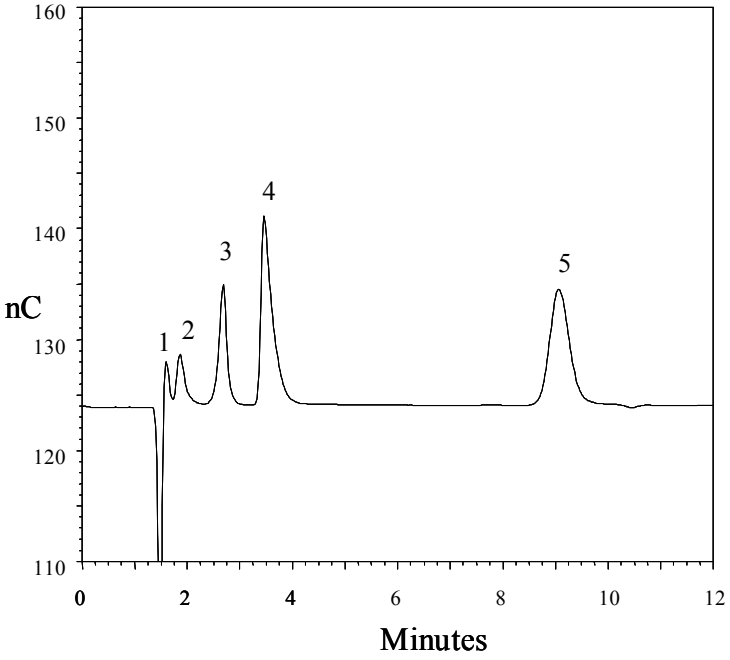
Column:	IonPac® ICE-AS1 (4 x 250 mm)
Temp.:	30 ° C
Eluent:	100 mM MSA
Flow Rate:	0.20 mL/min
Inj. Vol. :	20 µL
Det. Met.:	PAD
Electrode:	Disposable Pt (1 mm)
Gasket Thickness:	2 mil
Reaction Coil:	375 µL
Samples:	Standards (50 ppm, BuOH: 100 ppm)

Peaks:
1. Exclusion Volume, V_e
2. Sorbitol
3. Glycerol
4. Ethylene glycol
5. Methanol
6. Ethanol
7. 1-propanol
8. 1-Butanol

Figure 17 - Separation of Different Types of Alcohols

9.4.2. Separation of Chelating Agents

Until recently, sensitive ion chromatographic detection of carboxylic chelating agents required a post-column reaction with ferric nitrate. The new integrated amperometry procedure presented here makes possible a direct and sensitive detection of carboxylic chelates without any detection-enabling post-column procedure.



Column:	IonPac® AS7 (2 x 250 mm)
Temp.:	30 °C
Eluent:	75 mM MSA
Flow Rate:	0.20 mL/min
Inj. Vol. :	20 µL
Det. Met.:	PAD Waveform Table 10
Electrode:	Disposable Pt (1 mm)
Gasket Thickness:	2 mil
Samples:	NTA, EDTA, DTPA and EGTA (10 µM each)

Peaks:

1. Void
2. NTA (Nitrilotriacetic Acid)
3. EDTA (Ethylenediamine - Tetraacetic Acid)
4. DTPA (Diethylenetriamine - Pentaacetic Acid)
5. EGTA (Ethyleneglycol - bis (2-aminoethylether)_ - $N_1N_1N^1{}_1N^1$ - Tetraacetic Acid)

Figure 18 - Simultaneous Determination of Several Carboxylic Chelating Compounds

NOTE: Carbon electrodes are mostly used in DC amperometric detection mode. All electrodes will exhibit a steady decrease in signal output when used in the DC amperometric mode. Users may find that the rate of decrease will be lower with a disposable electrode than with a conventional working electrode. However, as with the conventional carbon electrodes (e.g. glassy carbon) the use of internal standards is recommended also with disposable carbon electrodes.

SECTION 10 – HPLC APPLICATIONS USING A CARBON ELECTRODE

NOTE: ***Use only glass containers for acidic eluents described in this section.***

10.1. Recommended Detection Conditions for HPLC applications (All potentials vs. Ag/AgCl)

+0.80 V (catecholamines); +1.00 V (phenol, nitrophenols, aminophenols and chlorophenols); +1.30 V (fat-soluble vitamins and antioxidants) and +0.70 V (benzidines).

Recommended settings for the data collection parameters in Chromeleon are as follows (for all chromatographic runs):

ED_1.Step = Auto
ED_1.Average = On
Data_Collection_Rate = 1 [Hz]

10.2. Part Numbers

The following part numbers refer to disposable and conventional carbon working electrodes. Additional part numbers are listed in Section 1.3. Recommended Dionex columns are specified in the figures.

ED (ICS-3000), ED40, ED50, and ED50A
069336 Carbon Disposable Electrodes, Pack of 6 with 6 (0.001") gaskets
069339 0.001" gasket.

ED (ICS-3000)
061753 ED Conventional Working Electrode, GC, with three 0.001" gaskets and polishing kit.

ED40, ED50, and ED50A
044115 Conventional Glassy Carbon Electrode with gasket and polishing kit.

10.3. Applications

For a more comprehensive presentation of example separations, please refer to the column manual of your specific column. The column manual also contains all experimental details for the separations listed below.

10.3.1. Separation of Catecholamines

High-performance liquid chromatography with electrochemical detection provides a direct, reliable and sensitive method for the determination of catecholamines. The example below shows the simultaneous analysis of norepinephrine (NE), epinephrine (E), dopamine (DA) and DHBA (internal standard) in human blood plasma. The plasma sample was processed with ClinRep® Sample Preparation Kit (Order Number 1000, Recipe GmbH, Munich, Germany, US Distributor: IRIS Technologies International, Roswell, GA).

10.3.1.2. Preparation of Standards and Eluents

10.3.1.2.1. Preparation of 3, 4-Dihydroxybenzylamine (DHBA) Internal Standard (from DHBA hydrobromide, Figure 19).

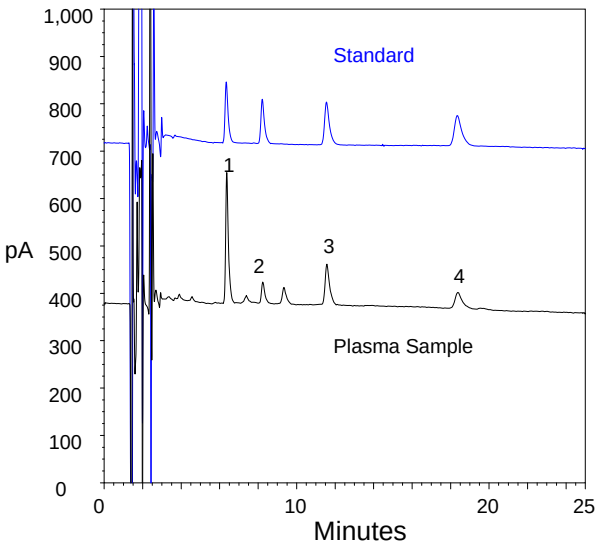
Prepare 0.10 M HCl from 37% concentrated HCl:

a. Into a 1.0 liter volumetric flask, add 8.3 ml of concentrated HCl and fill to the line with water. Store in a labeled container for future use. Shelf life is one year.
b. Prepare a DHBA concentrate (1 mM DHBA) by accurately weighing 0.022g DHBA and dissolving it into 100.0 g of 0.10 M HCl. Store in a refrigerator for up to six months.

c. Prepare an intermediate concentrate (10 µM DHBA) by accurately mixing 1.000 g of DHBA concentrate and 99.0 g of 0.10 M HCl. Store in a refrigerator for up to 3 months.
d. Prepare a DHBA test standard (100 nM DHBA) by accurately mixing 1.000 g of DHBA concentrate and 99.0 g of 0.10 M HCl. Store in a refrigerator for up to two weeks.

10.3.1.2.2. Preparation of Eluent

a. In a 1 liter dedicated bottle (with markings), mix 11.98 g of citric acid monohydrate, 3.53 g of anhydrous sodium acetate, 37.2 mg of ethylenediaminetetraacetic acid (EDTA, disodium form) and 10 mL of 100 mM octanesulfonic acid (OSA) with approximately 850 mL of DI water.
b. Stir or shake the buffer well to obtain a clear solution and vacuum filter through a 0.20 µm Nylon membrane (disposable filtration units Nalgene Cat. No.: 164-0020).
c. To the fitrate, add 100.0 mL of methanol and fill up to 1000.0 mL with DI water. The resulting eluent contains 57 mM citric acid / 43 mM Sodium Acetate / 0.10 mM EDTA / 1 mM OSA / 10% MeOH.



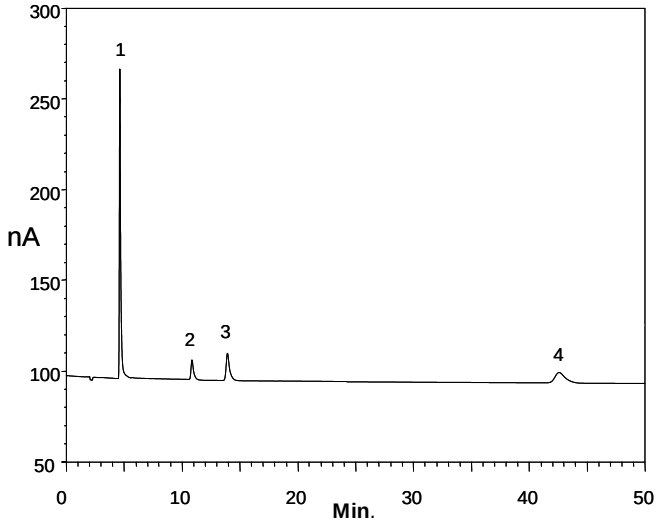
Column:	Acclaim C18 (2.1 x 150 mm)
Temp.:	25 ° C
Eluent:	Citric Acid / NaAc / EDTA / OSA / MeOH (pH 3.4)
Flow Rate:	0.20 mL/min
Inj. Vol. :	10 µL
Det. Met.:	DC (0.80 V vs. Ag/AgCl)
Electrode:	Disposable C
Gasket Thickness:	1 mil
Sample:	Standards (10 nM) and plasma sample

Peaks:
1. Norepinerphrine (NE)
2. Epinerphrine (E)
3. Dihydroxylbenzylamine (DHBA)
4. Dopamine (DA)

Figure 19 - Separation of Catecholamines: Standard and Human Blood Plasma Sample

10.3.2. Separation of Phenols

The separation of various types of phenols is achieved with a Dionex Acclaim 120 C18 column. The DC Amperometric detection of phenols requires a relatively high potential of +1.0V vs. Ag/AgCl. The example below shows a simultaneous analysis of 2-aminophenol, phenol, 4-nitrophenol and 4-chlorophenol.



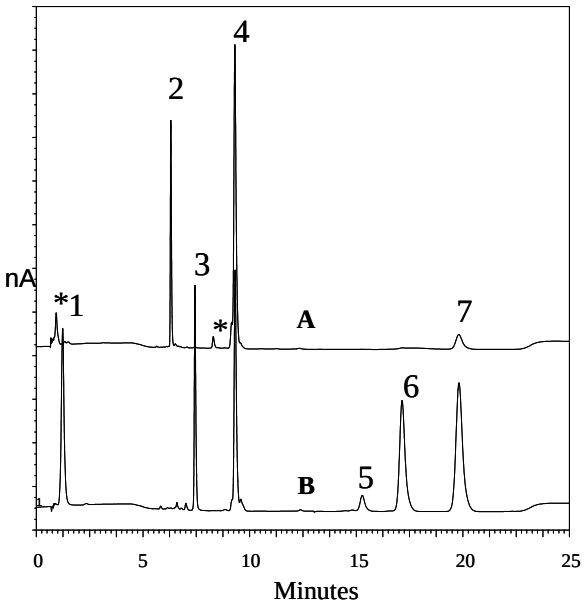
Column:	Acclaim® 120 C18 (2.1 x 150 mm)
Temp.:	30 ° C
Eluent:	MeOH / water (30:70), 20 mM NH_4Ac
Flow Rate:	0.17 mL/min
Inj. Vol. :	10 µL
Det. Met.:	DC (+1.0 V vs. Ag/ AgCl)
Electrode:	Disposable Carbon
Gasket Thickness:	1 mil
Sample:	Standards

Peaks:	1. 2-Aminophenol	25 µM
	2. Phenol	25 µM
	3. 4-Nitrophenol	25 µM
	4. 4-Chlorophenol	25 µM

Figure 20 - Separation of Various Types of Phenols

10.3.3. Separation of Antioxidants and Fat-Soluble Vitamins

The simultaneous analysis of antioxidants and fat-soluble vitamins is performed with a Dionex Acclaim 120 C18 column. Fat-soluble vitamins have been analyzed for many purposes in a variety of sample matrices. Among the most important assays are naturally occurring vitamins in food and beverages, vitamins added to nutrients as dietary supplements and vitamins in biological samples. Antioxidants can be found in vegetables together with fat-soluble vitamins. The DC amperometric detection with disposable a carbon electrode from Dionex provides a reliable and sensitive method for the determination of antioxidants and fat-soluble vitamins. The example below shows the simultaneous analysis of kaempferol (an antioxidant) and a selection of fat-soluble vitamins.



Column:	Acclaim® 120 C18 (2.1 x 100 mm)
Temp.:	30 ° C
Eluent:	E1: MeOH / water (94:6, v/v), 5 mM NaAc; E2: MeOH.
	0-1.3 min: 80%E1/20%E2; 1.4-19 min: 100%E1; 19.1-25 min: 80%E1/20%E2.
Flow Rate:	0.30 mL/min
Inj. Vol. :	10 µL
Det. Met.:	DC (+1.30 V vs. Ag/AgCl)
Electrode:	Disposable Carbon
Gasket Thickness:	1 mil
Sample:	Standards

Peaks:
1. Kaempferol (0.10 µM); 2. Butylated Hydroxytoluene;
3. Retinol (1 µM); 4. Retinyl Acetate (1 µM);
5. Cholecalciferol (4 µM); 6. γ -Tocopherol (1 µM);
7. α -Tocopherol (1 µM); * Unknowns.

Figure 21 - (A) multi-vitamin sample (2.5 mg /ml); (B) a standard mixture of five vitamins and kaempferol.

NOTE: ***Carbon electrodes are mostly used in DC amperometric detection mode. All electrodes will exhibit a steady decrease in signal output when used in the DC amperometric mode. Users may find that the rate of decrease will be lower with a disposable electrode than with a conventional working electrode. However, as with the conventional carbon electrodes (e.g. glassy carbon) the use of internal standards is recommended also with disposable carbon electrodes.***

SECTION 11 – IC APPLICATIONS USING A CARBON ELECTRODE

NOTE: ***Use only glass containers for acidic eluents described in this section.***

11.1. Quality Assurance

NOTE: ***Quality Assurance Reports are shipped with each order of disposable electrodes.***

The specified lifetime of disposable carbon electrodes for IC applications is two weeks. This specification is valid only under the analysis conditions in the QAR for P/N 069336. Lifetime specifications for disposable electrodes have been developed from long term experiments under carefully controlled conditions. Actual lifetime may vary depending on additional parameters not included in the long term experiments carried out at Dionex.

11.2. Recommended Detection Conditions for IC applications (All potentials vs. Ag/AgCl)

+1.35 V (S-Containing Amino Acids and Selective Detection of Peroxides over Alcohols); +1.4 V electroactive DNA bases, derivatives of electroactive DNA bases and DNA analogue drugs, for example acycloguanosine.

NOTE: ***We have found that 30-minute activation at +1.55 V can help to improve the signal stability for the detection of S-containing amino acids.***

We recommend setting the data collection parameters in your Chromeleon program as follows for all chromatographic runs:

ED_1.Step = Auto
ED_1.Average = On
Data_Collection_Rate = 1 [Hz]

11.3. Part Numbers

The part numbers for disposable and conventional carbon working electrodes are listed below. Additional part numbers are listed in Section 1.3. Recommended Dionex columns are specified in the figures throughout this document.

ED (ICS-3000), ED40, ED50, and ED50A
069336 Carbon Disposable Electrodes, Pack of 6 and six 0.001” gaskets.
069339 0.001” gasket.

ED (ICS-3000)
061753 ED Conventional Working Electrode, GC, with three 0.001” gaskets and polishing kit.

ED40, ED50, and ED50A
044115 ED50 Conventional Glassy Carbon Electrode with gasket and polishing kit.

11.4. Applications

For a more comprehensive presentation of separation examples, please refer to the column manual for your specific column. The column manual also contains all the experimental details for the separations listed below.

11.4.1. Separation of Sulfur-Containing Amino Acids

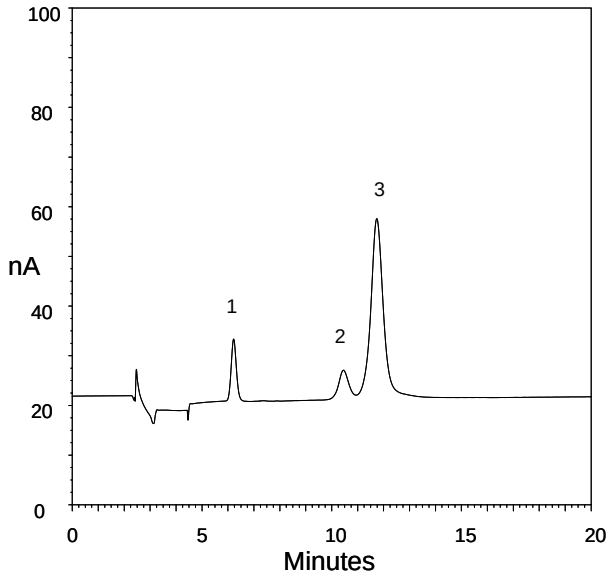
S-containing amino acids are widely studied because of their important role in metabolic processes. Some of the S-containing amino acids are considered to be biomarkers for certain diseases. The simultaneous separation of S-containing amino acids is achieved with an OmniPac PCX-500 column. The DC amperometric detection requires a disposable carbon electrode. The example below shows the simultaneous analysis of cysteine, homocysteine and methionine.

11.4.1.1. Preparation of the Methionine Standard (Met, FW 149.21, one of the S-containing amino acids in Figure 22)

a. Prepare 0.10 M HCl from 37% HCl: Add 8.3 ml of concentrated HCl into a 1.0 liter volumetric flask, and bring it up to 1.0 liter volume with water. Store in a labeled container for future use. Shelf life is one year.
b. Prepare a methionine concentrate,1 mM Met. Accurately weigh 0.0149 g Met and dissolve in 100.0 g of 0.10 M HCl. Store in a refrigerator for up to six months.
c. Prepare a 10 µM methionine test standard. Accurately mix 1.000 g of Met concentrate and 99.0 g of DI water. Store in the refrigerator for up to three months.

11.4.1.2. Preparation of Eluent

a. In a 1 liter dedicated bottle, dissolve 10.74 g of anhydrous monobasic sodium phosphate with approximately 850 mL of water.
b. Stir or shake well to obtain a clear solution and vacuum filter through a 0.20 µm Nylon membrane (disposable filtration units Nalgene Cat. No.: 164-0020).
c. Add 1.199 g of phosphoric acid (85%), 100.0 mL of methanol and fill up to 1000.0 mL with DI water. The resulting eluent contains 100 mM phosphate buffer (pH 3.0) / 10% MeOH.



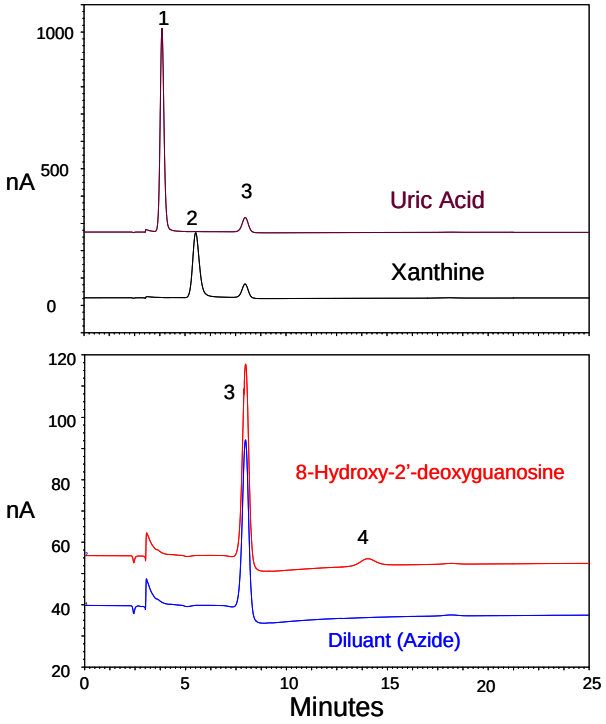
Column:	OmniPac PCX-500 (Anal. 2.0 x 250 mm; guard: 2.0 x 50 mm)
Temp.:	30 ° C
Eluent:	0.10 M Phosphate buffer / 10% MeOH (pH 2.0)
Flow Rate:	0.25 mL/min
Inj. Vol. :	25 µL
Det. Met.:	DC (1.35 V vs. Ag/AgCl)
Electrode:	Disposable Carbon (1 mm)
Gasket Thickness:	1 mil
Sample:	Standards (10 µM)

Peaks:
1.Cysteine
2. Homocysteine
3. Methionine

Figure 22 - S-Containing Amino Acids

11.4.2. Separation of Derivatives of Guanine

Some derivatives of guanine (a DNA base) can be separated with an OmniPac PCX-500. and detected with disposable carbon electrodes.



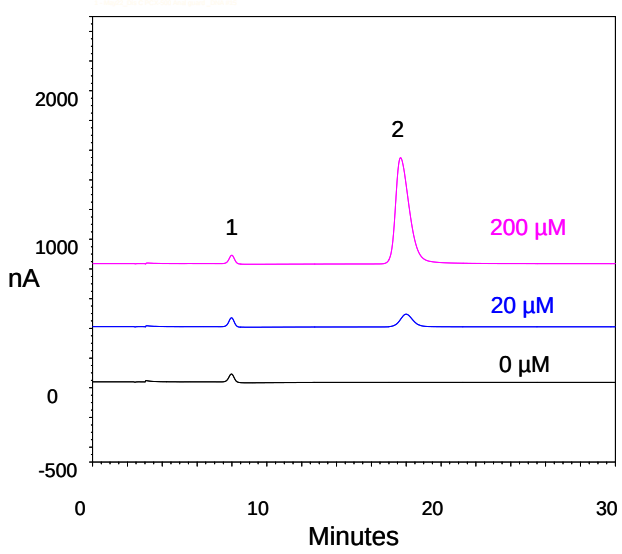
Column:	OmniPac® PCX-500 (Anal.: 2.0 x 250 mm; Guard: 2.0 x 50mm)
Temp.:	30 ° C
Eluent:	0.10 M phosphate buffer / MeOH (5%, pH 2.0)
Flow Rate:	0.25 mL/min
Inj. Vol. :	20 µL
Det. Met.:	DC (1.40 V vs. Ag/AgCl)
Electrode:	Disposable C (1 mm)
Gasket Thickness:	1 mil
Sample:	Standards (Uric acid, Xanthine, 8-Hydroxy-2'-deoxyguanosine and Azide diluant)

Peaks:	Concentration (µM)
1.Uric acid	50
2. Xanthine	50
3. Azide	
4. Hydroxy-deoxyguanosine	1

Figure 23 – Detection of guanine derivatives

11.4.3. Separation of DNA Analog Drug (Acycloguanosine)

The analysis of some DNA analog drugs can be done with the help of DC Amperometric detection using the same separation conditions as for the detection of guanine derivatives. The chromatogram below illustrates the detection of acycloguanosine (Zovirax or Zovir, GSK).



Column:	OmniPac®PCX-500 (Anal.: 2.0 x 250 mm; Guard: 2.0 x 50mm)
Temp.:	30 ° C
Eluent:	0.10 M phosphate buffer / MeOH (5%, pH 2.0)
Flow Rate:	0.25 mL/min
Inj. Vol. :	20 µL
Det. Met.:	DC (1.40 V vs. Ag/AgCl)
Electrode:	Disposable Carbon (1 mm)
Gasket Thickness:	1 mil
Sample:	Acycloguanosine Standards (0, 20, and 200 µM)

Peaks:
1.Azide
2. Acycloguanosine

Figure 24 - Detection of DNA Analog Drugs

SECTION 12 – TROUBLESHOOTING

The followings are examples of troubleshooting instruction for gold electrodes, for general troubleshooting tips of your ED50A and ED electrochemical detectors please use the appropriate sections of the ED50A (Doc.# 031722) and ED manuals (Doc#065031). Dionex publishes the latest version of the manuals on their web site (www.dionex.com). For troubleshooting of the application, please look at the appropriate column manual. Each column manual has detailed troubleshooting advice.

NOTE: Appropriate background levels for each electrode type are listed in the Quality Assurance Report (QAR) shipped with the electrodes.

12.1. The Signal Readout Remains at 0.0 nC

If there is no response at the detector, most likely the DE is installed upside down with the metal layer on the opposite side from the flow channel. Check the orientation of the disposable electrode, and if necessary, reassemble the cell with the electrode correctly orientated with the metal pattern facing (exposed to) the liquid stream.

12.2. Signal Remains at 0.0 nC or Randomly Fluctuates Over a Wide Range (e.g. -50 - +100nC)

If the disposable electrode has been removed and re-installed, it is possible that the signal will remain at 0.0 nC or randomly fluctuate over a wide range (e.g. -50 to +100nC). If this is the case, the contact pad of the DE may have been damaged by the old-style (three-pointed) pogo. Open the cell and examine the disposable electrode for damage, such as a hole in the metal film through which the substrate can be seen. If this is the problem, it is important to first replace the old-style pogo with a new, blunt pogo (P/N 045939). Once the pogo has been replaced you can install a new DE.

12.3. Signal Increases Out of the Useful Range >1000 nC and Remains at a High Level

If the signal increases out of range, the working electrode portion of the DE (or portions of the working electrode) has probably lost adhesion with the polymeric substrate. Switch the cell voltage off immediately. Occasional loss of adhesion is normal if the electrode is used longer than the recommended period of time. However, please notify Dionex should this occur within the specified life-time of the electrode, and provide the lot number of the electrode, the waveform used, and the eluent composition.

To confirm that the electrode is the problem, remove the damaged electrode and confirm visually the lack of adhesion in the area of the working electrode. If the electrode is not visually dissociated from the substrate, gently spray the DE edges with clean dry air at a pressure not to exceed 20 psi. Watch to see if the DE lifts from the substrate. If the electrode still appears to be intact, cover the entire length of pad-lead-electrode pattern with Scotch Tape. After making sure that the tape is in good contact with the entire pattern, pull off the tape. An adhesion problem is indicated by portions of the pattern being removed from the DE surface.

NOTE: If the adhesion is still good and the pattern remains intact even after the Scotch tape application consider other possible causes such as: leaking working electrode gasket or a leak inside the reference electrode compartment.

12.4. Excessive Peak Tailing or Negative Peaks

The most likely cause of excessive peak tailing or negative peaks is incorrect values of potentials applied to the DE. Check the waveform that is actually being applied to the DE by reviewing the program file. If the applied waveform is correct, check the potential of the reference electrode against another reference electrode that was never exposed to alkaline eluents. If the potential difference of both electrodes immersed in 0.1 M KCl exceeds 30 mV, install a new reference electrode.

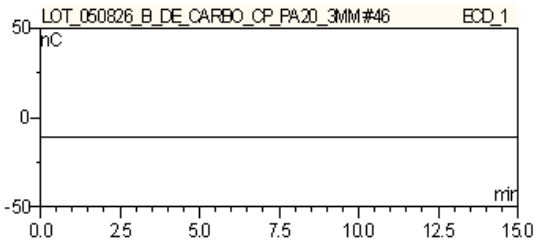
APPENDIX A – EXAMPLE QAR

Quality Assurance Reports (QARs) are shipped with each order. The following examples are for reference only.

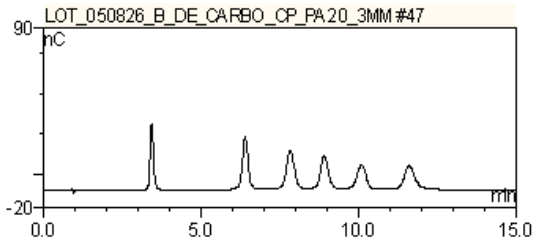
A1 - Carbohydrate Disposable Electrode (Product No. 060139 or 066480) Lot Validation

Carbohydrate Disposable Electrode
Product No. 060139
Lot Validation

Lot# 050826B **Date: 07-Oct**



Typical Initial Start Up (Drift)



Monostandards - Mix of Six

Chemicals for Carbohydrate Analysis

Dionex Monostandards Mix of Six 100 nmol – Dionex Product No. 043162
Sodium hydroxide, 50% Certified Grade – Fisher Scientific Catalog No. SS254
Use only 18.2 mΩ.cm deionized water for the preparation of eluents.

Analytical:	CarboPac ™ PA20 (3 X 150 mm)
Guard:	CarboPac PA20 (3 X 30 mm)
Flow Rate:	0.50 mL/min
Detection:	ED50
Pump:	GP50, without Degas
Temperature:	30 °C, using an AS50 Thermal Compartment
Autosampler:	AS50, using 1.5 mL glass vials/pre-cut septa
Injection Volume:	10 µL
Standard:	Monostandards Mix of Six
Diluted Standard:	10 nmol/mL in DI Water
	Each analyte is 0.1 nmoles per injection.

Eluent Composition

%A	200 mM NaOH
%B	10mM NaOH

Eluent Profile

Time	%A	%B	Comment
-25.00	100	0	Regeneration
-15.05	100	0	
-15.00	0	100	Equilibration
0.00	0	100	Inject
12.00	0	100	End

Quadruple Waveform

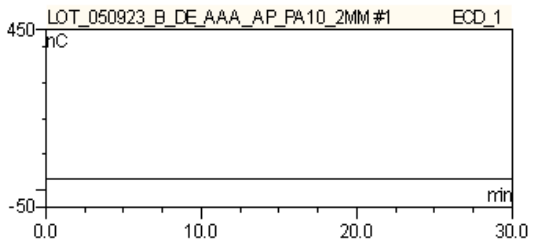
Time	Potential[1]	Integration
0.00	0.10	
0.20	0.10	Begin
0.40	0.10	End
0.41	-2.00	
0.42	-2.00	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	

[1] **Potential:** V vs. Ag/AgCl

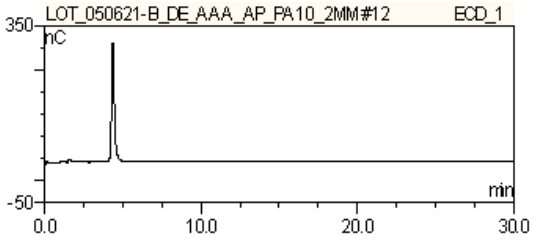
A2 - AAA Disposable Electrode (Product No. 060082) Lot Validation

AAA Disposable Electrode
Product No. 060082
Lot Validation

Lot#: 050621B **Date: 03-Aug**



Typical Initial Start Up (Drift)



Histidine Test Chromatogram

Chemicals for AAA Analysis

Dionex Sodium Acetate – Dionex Product No. 059326
Dionex L-Histidine Standard – Dionex Product No 059567
Sodium hydroxide, 50% Certified Grade – Fisher Scientific Catalog No. SS254
Sodium Azide – Sigma Catalog No. S8032
Use only 18.2 mΩ.cm deionized water for the preparation of eluents.

Analytical:	AminoPac ® PA10 (2 X 250 mm)
Guard:	AminoPac PA10 (2 X 50 mm)
Flow Rate:	0.25 mL/min
Detection:	ED50
Pump:	GP50, with Degas
Temperature:	30 °C, using an AS50 Thermal Compartment
Autosampler:	AS50, using 1.5 mL glass vials/pre-cut septa
Injection Volume:	25 µL
Standard:	8 µM Histidine

Eluent Composition

%A	18.2 mΩ.cm deionized water	20%
%B	250 mM NaOH	70%
%C	1M sodium acetate	10%

Waveform

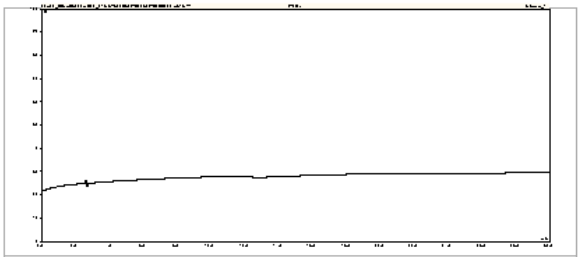
Time	Potential[1]	Integration
0.00	0.13	
0.04	0.13	
0.05	0.33	
0.21	0.33	Begin
0.22	0.55	
0.46	0.55	
0.47	0.33	
0.56	0.33	End
0.57	-1.67	
0.58	-1.67	
0.59	0.93	
0.60	0.13	

[1] **Potential:** V vs. pH/AgCl, 3 MKCl

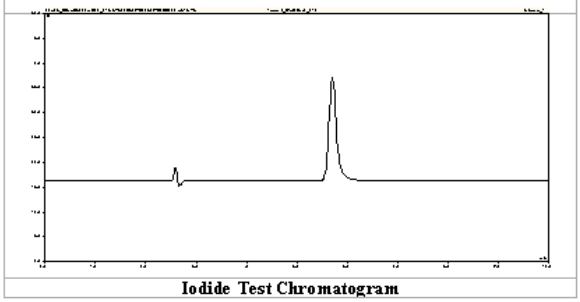
A3 - Disposable Silver Electrode (Product No. 06003) Lot Validation

Iodide Separation with IonPac® AS17

Lot#: YYMMDD **Date: Enter Test Date**



Typical Initial Start Up (Drift)



Iodide Test Chromatogram

Analytical:	IonPac AS16 (2 X 250)
Guard:	IonPac AS16 (2 X 50)
Flow Rate:	0.25 mL/min
Detection:	ED50
Pump:	GP50, with Degas
Temperature:	30 °C, using an AS50 Thermal Compartment
Autosampler:	AS50, using 1.5 mL glass vials/pre-cut septa
Injection Volume	10 µl
Standard:	1 ppm Iodide

Eluent Composition

%A	18.2 mΩ.cm deionized water	75%
%B	250 mM NaOH	25%

Waveform

Time (s)	Potential [1]	Current Integration
0.00	-0.1	
0.20	-0.1	Begin
0.90	-0.1	End
0.91	-1.0	
0.93	-0.30	
1.00	-0.30	

[1] **Potential:** V vs. pH/Ag/AgCl, 3 M KCI
Reference Electrode Mode: AgCl

Chemicals for Iodide Analysis with IonPac AS17 Column

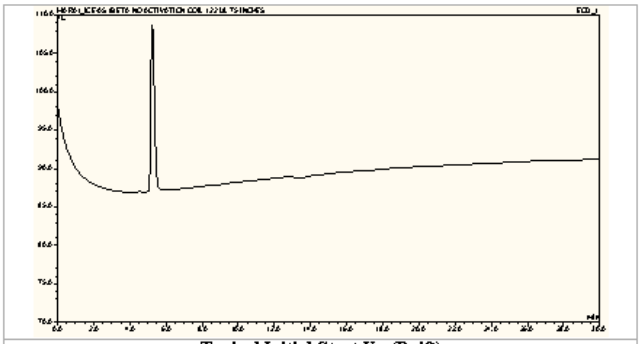
Sodium Iodide Standard – Aldrich Catalog No 38,311-2
Sodium hydroxide, 50% Certified Grade – Fisher Scientific Catalog No. SS254
Use only 18.2 mΩ.cm deionized water for the preparation of eluents.

A4 - Disposable Platinum Electrode (Product No. 06440) Lot Validation

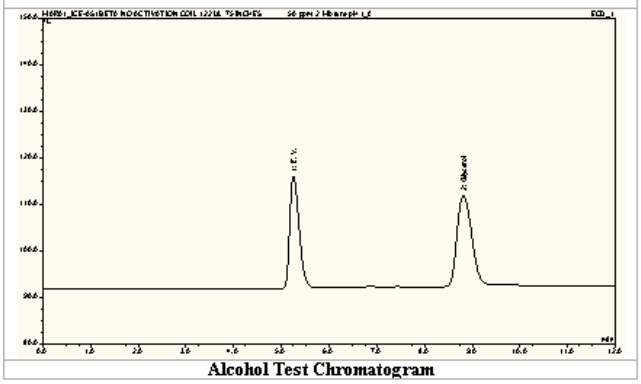
Glycerol Separation with IonPac® ICE AS1

Lot#: YYMMDD

Date: Enter Test Date



Typical Initial Start Up (Drift)



Alcohol Test Chromatogram

Analytical:	IonPac ICE-ASI (4 X 250)
Flow Rate:	0.20 mL/min
Detection:	ED50
Pump:	GP50, with Degas
Temperature:	30 °C, using an AS50 Thermal Compartment
Autosampler:	AS50, using 1.5 mL glass vials/pre-cut septa
Injection Volume	20 µl
Reaction Coil:	375 µL
Standard:	Glycerol (50 ppm)

Eluent Composition

%A	0.10 M Methanesulfonic acid	100%

Waveform

Time (s)	Potential [1]	Current Integration
0.00	0.30	
0.31	0.30	
0.32	1.15	
0.64	1.15	Begin
0.66	1.15	End
0.67	-0.30	
1.06	-0.30	
1.07	0.30	

[1] **Potential:** V vs. pH/Ag/AgCl, 3 M KCl
Reference Electrode Mode: AgCl

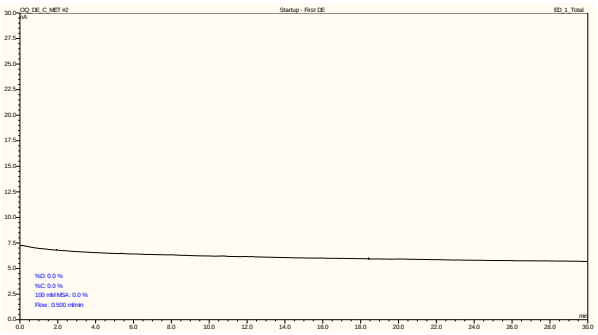
Chemicals for Alcohol Analysis with IonPac ICE-ASI Column

Glycerol – Aldrich Catalog No G2289-500 mL
Methanesulfonic Acid – Aldrich Catalog No. 471356-500 mL
Use only 18.2 mΩ.cm deionized water for the preparation of eluents.

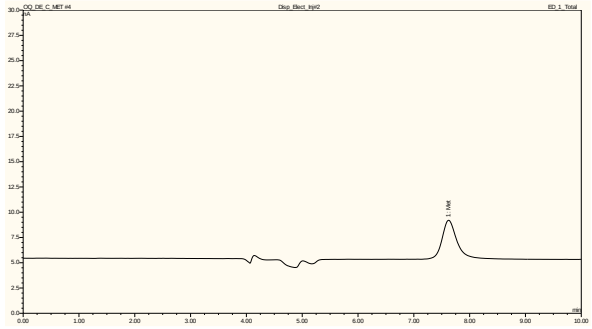
A5 - Disposable Carbon Electrode (Product No. 069336) Lot Validation

Methionine Separation with OmniPac® PCX-500

Lot #: YYMMDDDate: Enter Test Date



Typical Initial Start Up (Drift)



Met

Experimental Conditions:

Columns: **OmniPac® PCX-500 (Anal.: 4.0 X 250 mm; guard: 4 x 50 mm)**
Flow Rate: 0.50 mL/min
Detection: **+1.35V (vs. Ag/AgCl)***
Pump: ICS-3000 SP/DP
Temperature: **30 °C**
Autosampler: **AS, using 1.5 mL glass vials/pre-cut septa**
Injection Volume: **25 µl**
Standard: **methionine (10 µM)**

Eluent Composition

%A	100 mM phosphate buffer (pH 3.0) / 10% MeOH	100%

*: 30-minute activation at +1.55 V is used as the initial equilibration.

Chemicals for Methionine Analysis with OmniPac® PCX-500 Column

Sodium phosphate monobasic, anhydrous– Aldrich No. S0751-1000 g
Phosphoric acid – Aldrich Catalog No. 215104, 2kg
Methanol – Aldrich Catalog No. 646377, 4L
Methionine-Sigma Catalog No. M9625-5g,
Use only 18.2 mΩ.cm deionized water for the preparation of eluent

APPENDIX B – INSTALLATION AND START-UP USING ED40, ED50, AND ED50A CELLS

B.1 - Comparison of the Conventional Electrode Parts with the Disposable Electrode Parts

Before proceeding with the installation of your disposable electrodes, please take time to review the following two figures. These figures will provide you with a point of reference when comparing installation of your new disposable electrodes with the more familiar process of installing a conventional electrode.

Figure B-1 shows an ED40 cell body as it is normally configured for use with conventional electrodes. Figure B-2 shows a conventional silver electrode and two wing nut screws that are the standard components of each ED40 cell. Additionally, Figure B-2 also shows the disposable electrode (DE) and the modified gasket (MG). You will notice the small circle on the DE, which is the working electrode, the larger circle, which is a contact pad and the straight, narrow lead which connects them. The reverse side (the "dull" side) of the polyester sheet does not carry any metal.

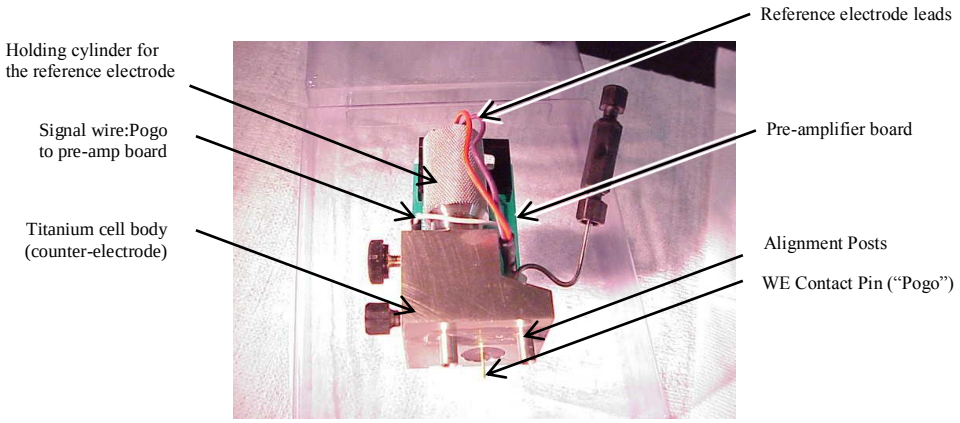


Figure B-1 - The Conventional Side
View of the ED 40 cell body, pre-amplifier board, and reference electrode holder

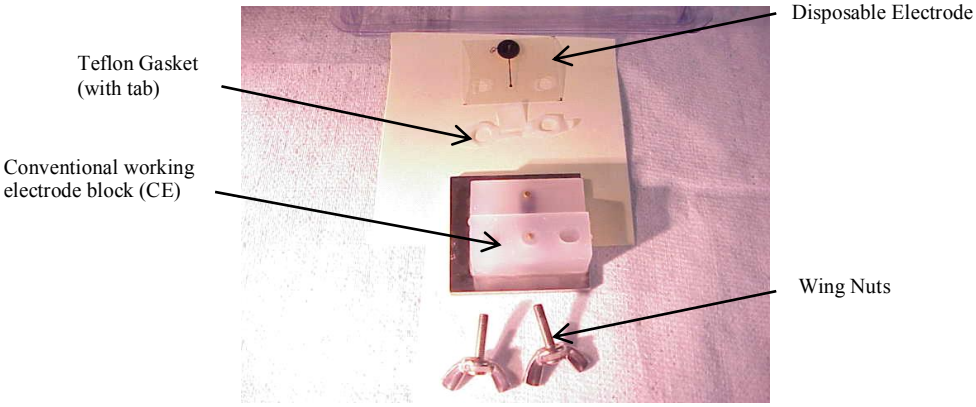


Figure B-2 – Comparison
Conventional Electrode, Disposable Electrode, and the new gasket required for Disposable Electrode usage

B.2 - Installation of a Blank Block into a Conventional Detection Cell

When using disposable electrodes with a cell that originally was configured with conventional electrodes a spacer block must be used instead to properly support the disposable electrode. Install a blank block (P/N 060297) prior to installation of the disposable electrode.

WARNING: ***INSTALLING THE DISPOSABLE ELECTRODE OVER THE CONVENTIONAL ELECTRODE MAY COMPROMISE THE DE LIFETIME.***

B.3 Installation of a Blunt Pogo into an "Old-Style" Detection Cell

The contact pins in older versions of ED40/50 cell (pre 2004) have three sharp points at the top and can damage the contact pad of a DE. The old pogo must be replaced with a new blunt pogo. Read the following steps completely before attempting installation of the blunt pogo.

NOTE: Be careful not to compress or bend the Pogo during the installation.

a. Compare the tip of the enclosed Pogo with the tip of that installed inside your cell.
b. If you determine the installed Pogo to be of the older type, with three sharp points, slip a 1 cm-long segment, approximately 2-mm I.D., of Tygon or Teflon tubing over the old Pogo.
c. Using pliers of suitable size, pull the old Pogo out of the cell, holding it where it is protected by the soft tubing.
d. Slip the soft tubing over the upper part of the new blunt Pogo
e. Holding the tubing protected segment with pliers, insert the blunt Pogo into the cell.

B.4 Installation of a Disposable Electrode into a Cell

If you are installing the disposable electrode into an existing electrochemical cell that previously held a conventional working electrode, please read sections B.2 and B.3 before proceeding. Figures B-3 through B-9 show the proper assembly and show all parts from Figures B-1 and B-2 in a single picture.

WARNING: ***WEAR GLOVES WHEN HANDLING THE DE. FINGERPRINTS CAN CONTAMINATE THE ELECTRODE AND DECREASE PERFORMANCE.***

Remove the regular electrode and install a blank polypropylene block to mount the disposable electrode and modified gasket inside the electrode cell

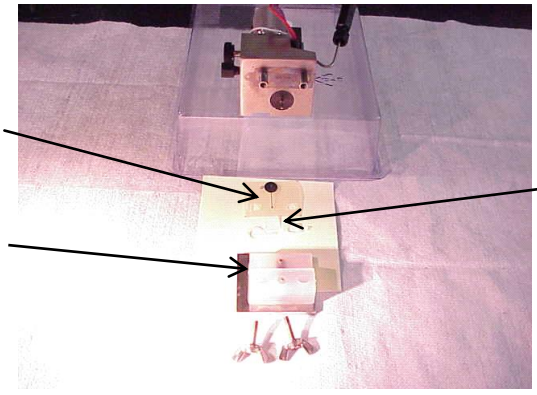


Figure B-3 - Overview of Parts Used During Disposable Electrode Installation

NOTE: Notice the proper position of the "skirt." This is an important step.

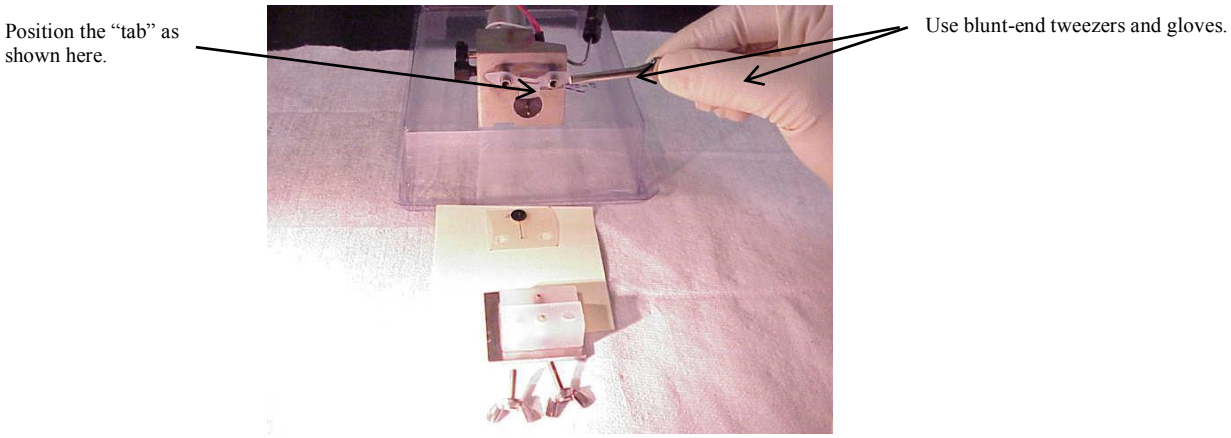


Figure B-4 - Installing the Modified Gasket

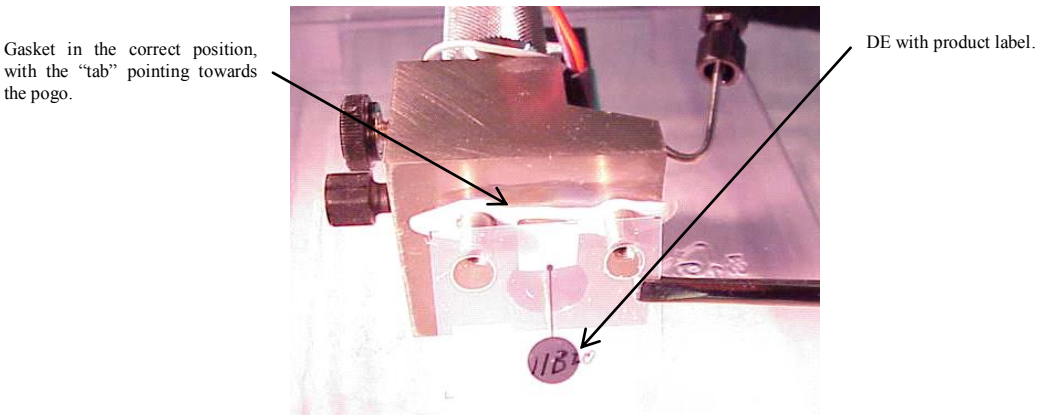


Figure B-5 - Installing the DE

The "bright" side of the DE is the active side and faces the gasket and the cell body. The "dull" side is recognizable by label and must face away from the cell body.

The working electrode is positioned in the center of the flow path. The flow path is defined by the Teflon Gasket cutout. The "tab" provides additional sealing for the lead between the working electrode and the circular contact pad.

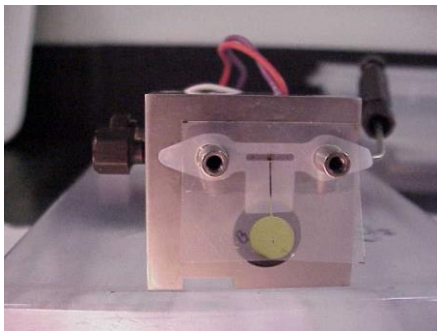


Figure B-6 - Electrode Positioning

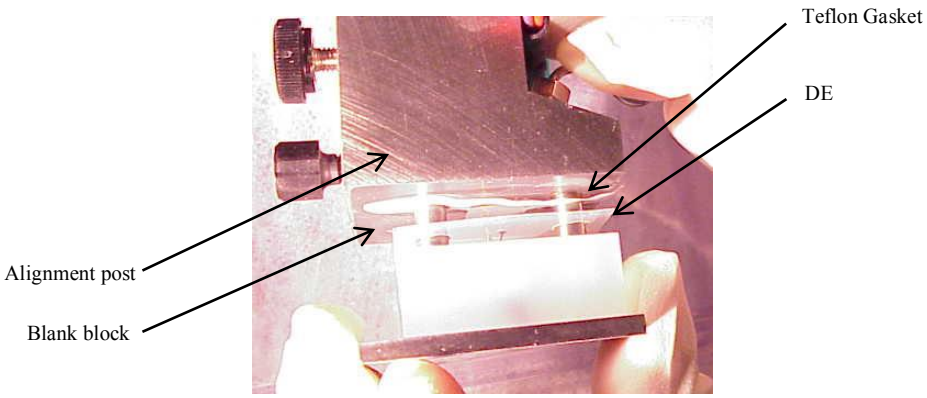


Figure B-7 - Placing CE onto the Alignment Posts

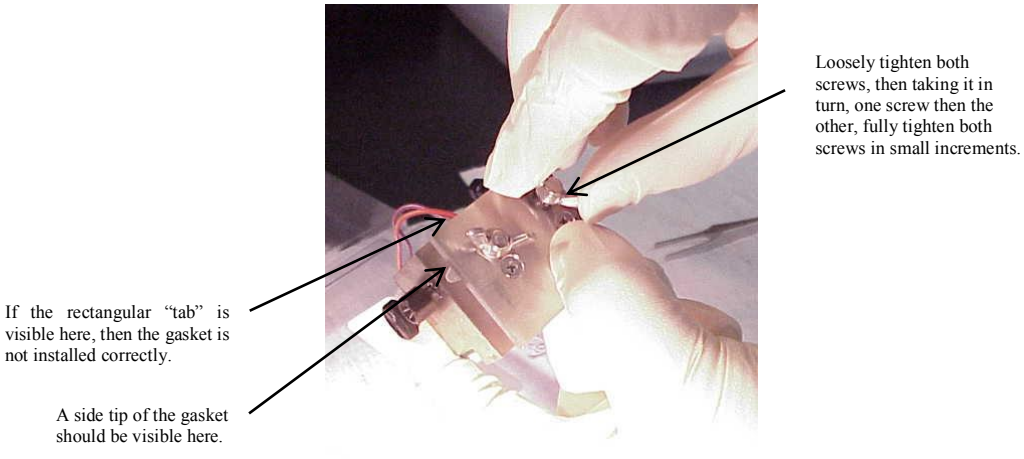


Figure B-8 - Final Stage of Cell Assembly
Tightening the Wing Nut Screws

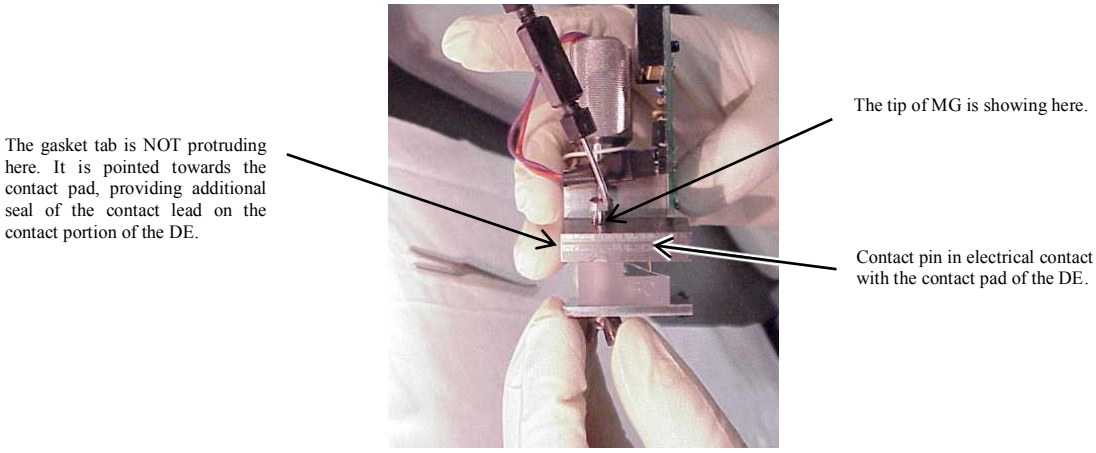


Figure B-9 - The DE Installed in the ED40/50/50A Cell