

Carbohydrate Analysis Workshop Tips & Tricks

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Recommendations from the Experts in HPAE-PAD

What Compounds Can Be Separated by HPAE?

- Must be anionic, preferably at high pH
- If analyzed at high pH, the compound should be stable at high pH*
- Compounds that are anionic at neutral pH will require acetate in the eluent
- If separated at neutral pH, NaOH must be added postcolumn for PAD detection

*Determining the degradation product or the remaining compound may be acceptable.

What Compounds Can Be Detected by PAD*?

- Compounds must have hydroxyl groups (alcohols are detected, but with poor sensitivity)
- Compound should be stable at high pH
- If the compound is unstable at high pH, separate at neutral or low pH and add NaOH postcolumn for PAD detection
- Check detection by performing a flow injection analysis

* Under carbohydrate analysis conditions

Carbohydrates That Cannot Be Determined by HPAE-PAD

- O-Methylated oligogalacturonic acids*
- O-Acetylated sialic acids*
- Pinitol (if determining together with inositol)
- Compounds that contain hydroxyls, but have more than two charged substituents (phosphates, carboxylates, sulfates)
- Some synthetic sugars in which many of the hydroxyl groups have been substituted

*Can be separated at neutral pH and detected after postcolumn addition of NaOH.

Carbohydrates: Matrix Interferent, Effect, and Possible Removal

Matrix Interferent	Effect	Possible Removal
Hydroxylated compounds (e.g. Tris buffers, alcohols)	PAD-active (interferes with carbohydrate detection)	Dialysis or dilution
Halides	Will bind to column, may affect retention time of analytes and interact with the gold electrode.	Dialysis, dilution, or solid-phase extraction using OnGuard-Ag (silver) cartridge.
Amine-containing compounds (including proteins, peptides and free amino acids)	PAD active	Solid-phase extraction using OnGuard-A (anion-exchange). For inline use, the AminoTrap column is used for proteins, peptides, and amino acids
Lipids	May damage column	Liquid-liquid extraction or supercritical fluid extraction.
Organic Solvents	May affect analyte retention and cause diminished electrode response	Solid phase extraction using OnGuard RP (reversed phase)
Anionic detergents (such as SDS)	Will bind irreversibly to the column	Solid phase extraction using OnGuard RP

Sample matrices in glycoprotein analysis can be greatly simplified by performing a Western blot and selectively removing the carbohydrates from the PVDF membrane-bound proteins. Refer to DIONEX Technical Note 30, "Monosaccharide and Oligosaccharide Analysis of Glycoproteins Electrotransferred onto Polyvinylidene Fluoride (PVDF) Membranes" on the Dionex Reference Library CD-ROM.

Thermo Scientific Dionex CarboPac™ Columns combined product manual (Doc. #: 031824-06)

Good Practices for Successful HPAE-PAD

- Always use a guard column
- Keep tubing lengths to a minimum and change tubing from the injector to the column, between columns, and to the detector at least once a year
- Change the sample loop at least once a year. Remember to calibrate the loop for proper comparison to the last loop.
- Change the autosampler needle every six months to a year

Good Practices for Successful HPAE-PAD-II

- Develop all new methods with Waveform A (TN 21)
- Change the reference electrode every 3–6 months and be sure to calibrate it
- Disconnect the column from the cell when cleaning the column
- Control column and cell temperature at 30 °C

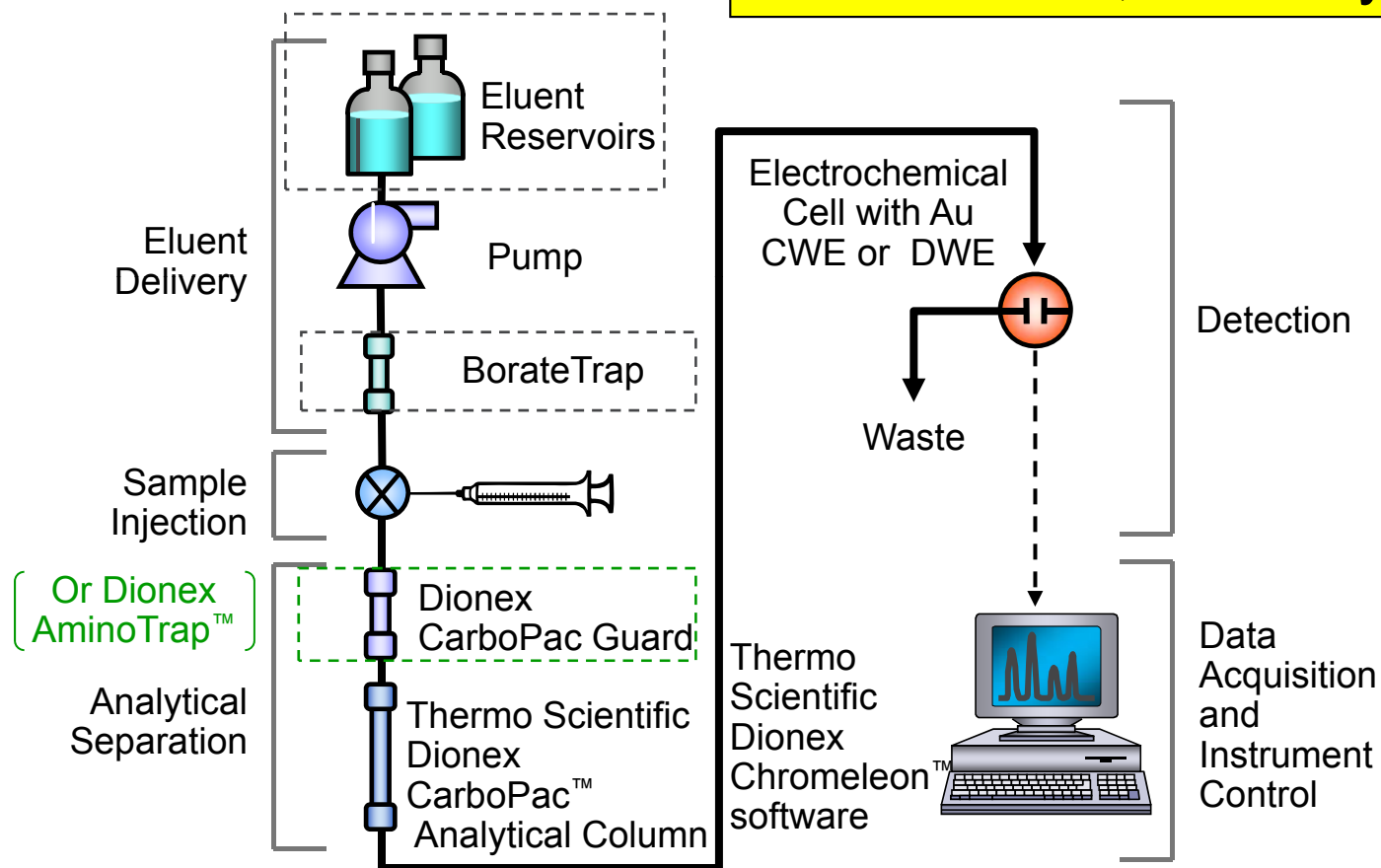
HPAE-PAD Parameters to Follow

- System backpressure
- Background (20–35 nC for Waveform A and degas)
- Baseline noise (30–100 pC peak to peak)
- Reference electrode pH (must be calibrated)*
- For monosaccharide analysis, mannose asymmetry

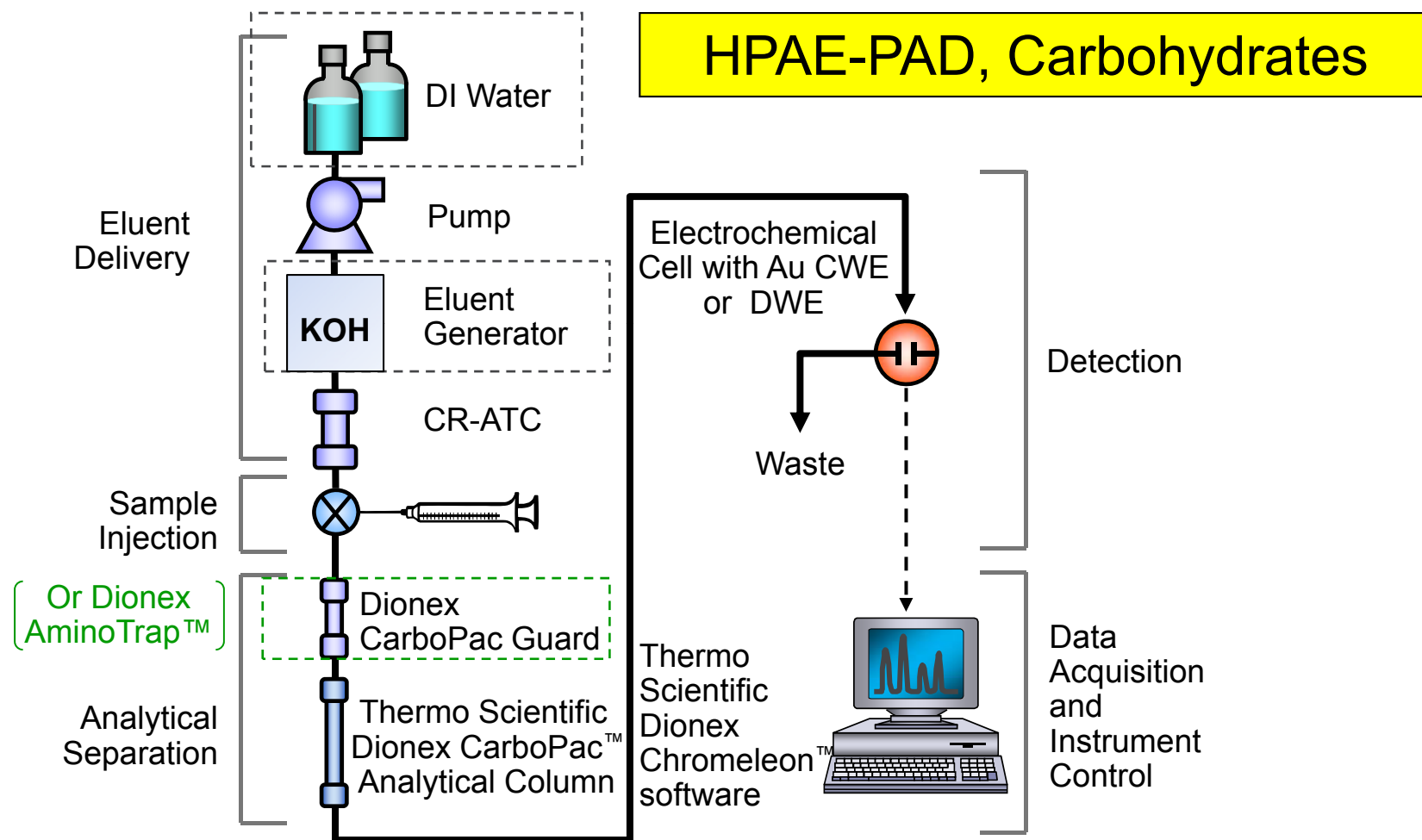
* A reference electrode that does not give a pH reading and can not be calibrated is no good. It should only be calibrated once.

System Hardware — Manually-Prepared Eluent

HPAE-PAD, Carbohydrates



System Hardware — Electrolytically-Generated Eluent



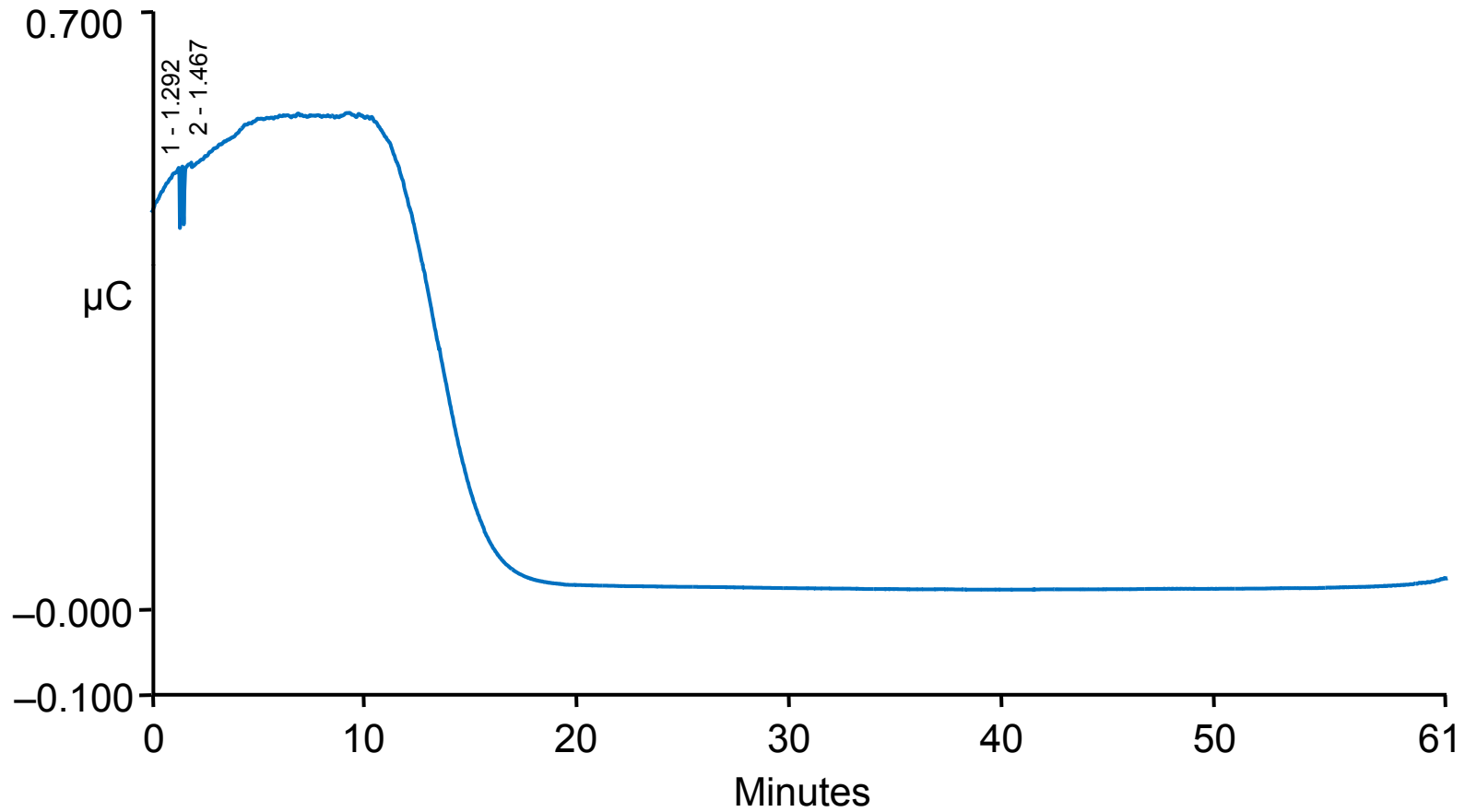
Water Quality for HPAE-PAD

- Use high quality, 18 M Ω -cm deionized water
- Water should be free of bacteria and mold (this will be a bigger problem for hydrolysis)
- The deionized water system should use cartridges that are free of glycerol (some are packed in glycerol)
- Water may not be useful a few days after a peroxide disinfection (water system should be well flushed)
- Bottled HPLC-grade water (with the exception of Burdick and Jackson) should not be used because most bottled water contains an unacceptable level of electroactive impurities
- Peak efficiencies will be diminished (especially for mannose and alditols) if the eluent water contains >10 $\mu\text{g/L}$ (10 ppb) borate

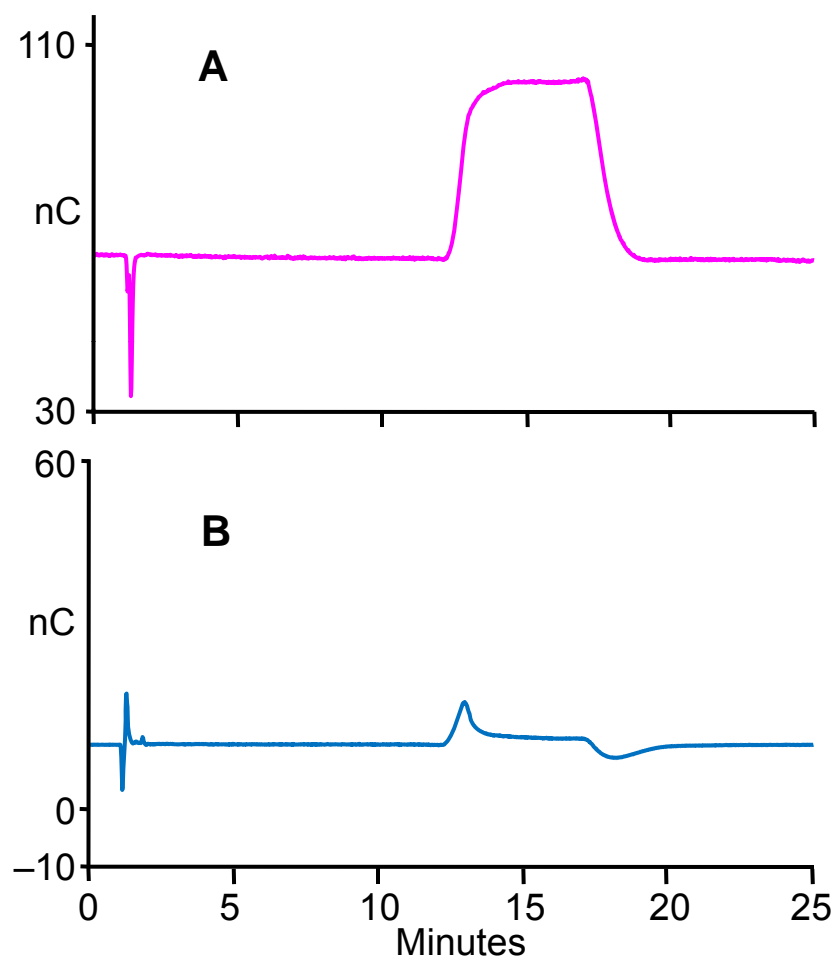
New System Start-Up — System Rinse

1. Rinse a new system with 2 M NaOH prior to use
2. DO NOT install the Thermo Scientific Dionex CarboPac™ column before confirming that the background < 30 nC
3. Place the 2 M NaOH in a prerinsed bottle and place all four eluent lines in it. Withdraw at least 40 mL of NaOH from each line using a syringe.
4. Close the solvent draw-off valve and leave the pump proportioning 25/25/25/25 for 15 min
5. Make sure that all surfaces come into contact with the NaOH; rotate the injection valve
6. Repeat the process with 18.2 MΩ-cm DI water
7. If using an eluent generator, rinse with 100 mM KOH for 2 h

Effect of Residual Peroxide on HPAE-PAD Response



Glycerol in the Eluent Water

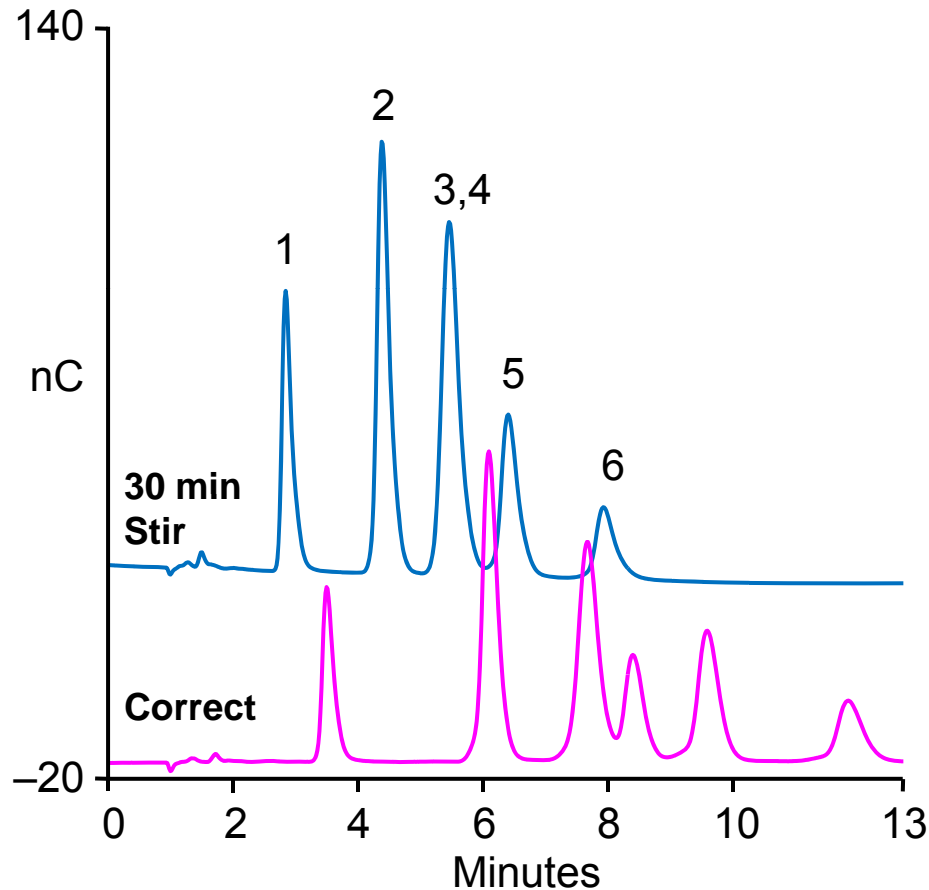


Column: Thermo Scientific Dionex CarboPac™ PA1 + guard
Eluent: 0–12 min, 150 mM Sodium hydroxide
150 mM sodium acetate;
12–17 min, 150 mM sodium hydroxide
500 mM sodium acetate;
17–25 min, 150 mM sodium hydroxide
150 mM sodium acetate
Flow Rate: 1.0 mL/min
Inj. Volume: 25 μ L
Temperature: 30 °C
Detection: PAD waveform B (TN 21)
Sample: Water
Chromatograms: A) Water with glycerol from DI cartridges
B) Good water

Manual Preparation of NaOH Eluents

- Use a plastic serological pipet to add the appropriate amount of 50% NaOH (5.2 mL for 1 L of 100 mM) to water. Remove the NaOH from the middle of the bottle and do not shake the bottle. Swirl to mix.
- **DO NOT USE NaOH PELLETS!**
- Vacuum degas the water for best reproducibility
- Store under helium. Prepare new eluent if >1 week old
- If helium headspace is lost, prepare new eluent

Monosaccharide Analysis: Manually-Prepared Eluents — Carbonate Contamination



Column: Thermo Scientific Dionex CarboPac™ PA20 + Thermo Scientific Dionex AminoTrap™

Eluent: 10 mM NaOH

Flow Rate: 0.5 mL/min

Inj. Volume: 10 µL

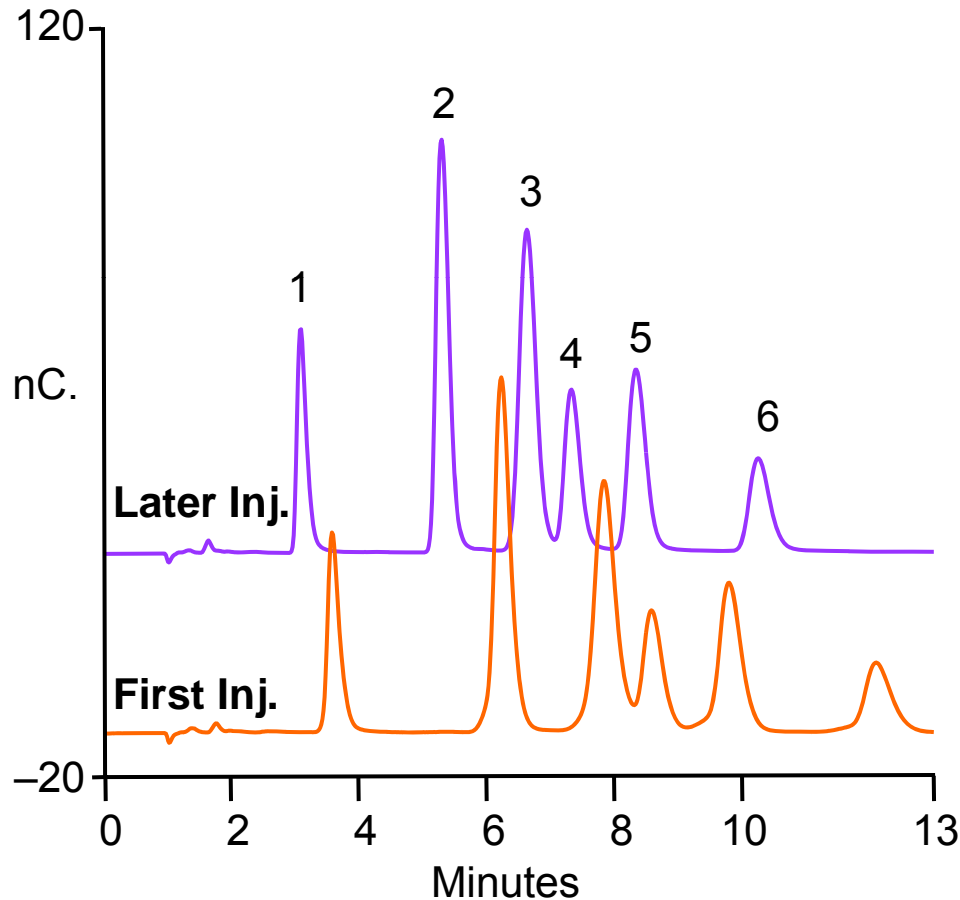
Detection: PAD (Au) disposable
Waveform A (TN 21)

Temperature: 30 °C

Sample: Mix of six standard (50 µM)

Peaks:	1. Fucose	500 pmole
	2. Galactosamine	500
	3. Glucosamine	500
	4. Galactose	500
	5. Glucose	500
	6. Mannose	500

Monosaccharide Analysis: Manually-Prepared Eluents — No Column Regeneration



Column: Thermo Scientific Dionex CarboPac™ PA20 + Thermo Scientific Dionex AminoTrap™

Eluent: 10 mM NaOH

Flow Rate: 0.5 mL/min

Inj. Volume: 10 µL

Detection: PAD (Au) disposable waveform A (TN 21)

Temperature: 30 °C

Sample: Mix of six standard (50 µM)

Peaks:

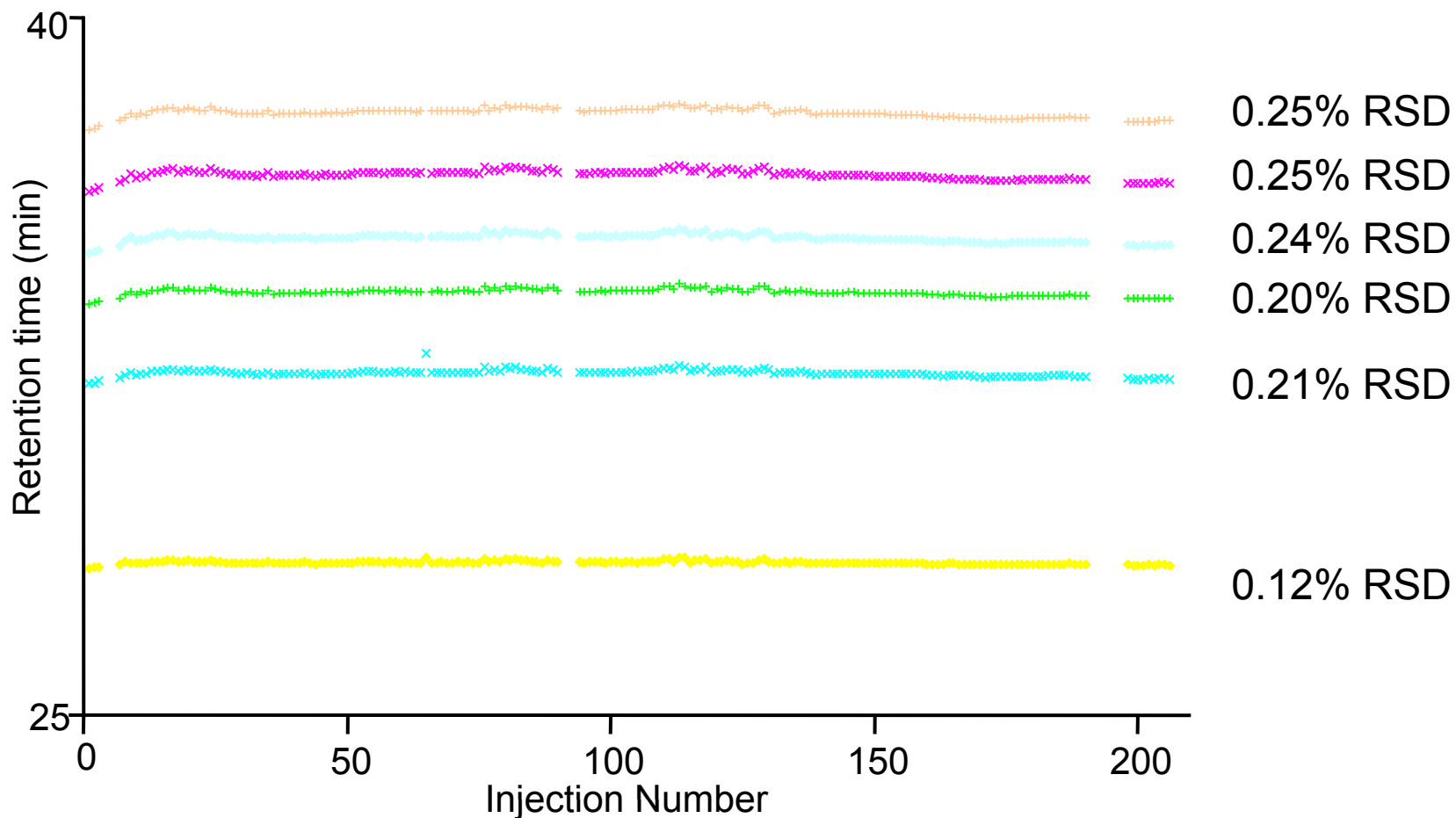
1. Fucose	500 pmole
2. Galactosamine	500
3. Glucosamine	500
4. Galactose	500
5. Glucose	500
6. Mannose	500

Advantages of Using an Eluent Generator for HPAE-PAD

- Requires only deionized water as the carrier
- Generates high-purity, carbonate-free hydroxide eluent on-line
- KOH concentration from 0–100 mM (analytical and microbore) or 0–200 mM (capillary), controlled electrically with minimal delay
- Improved resolution, retention time, and area RSD
- Minimizes pump maintenance—pumps only have contact with DI water

EG — Eluent Generator Retention Time RSDs

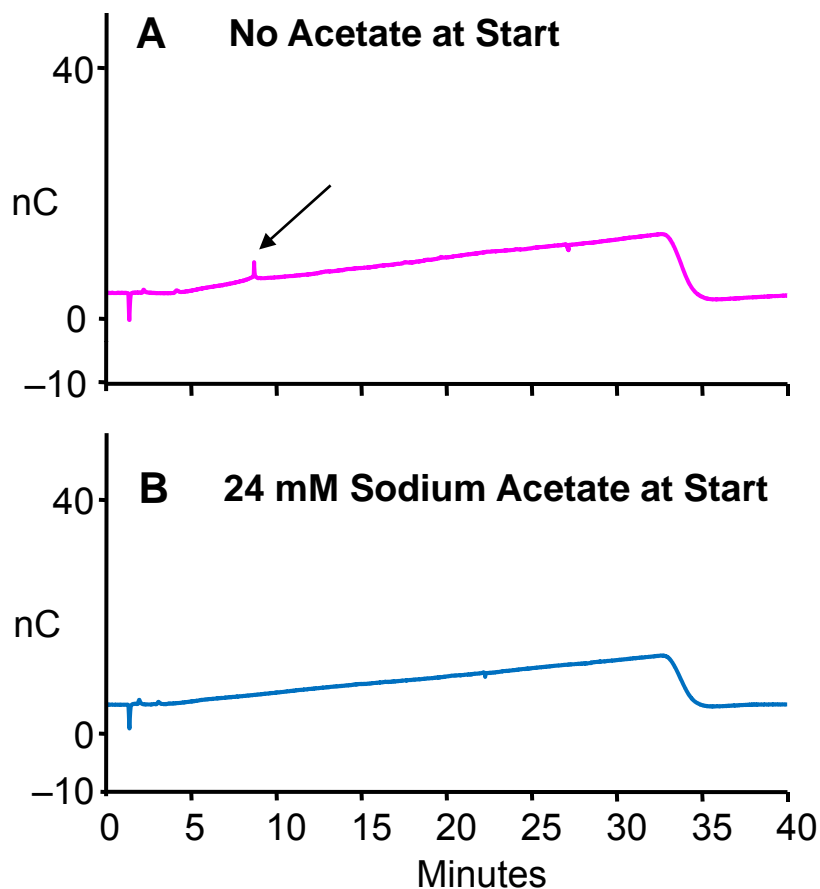
Long-Term Reproducibility of Monosaccharide Standards



Preparation of Sodium Acetate Eluents

- Use the highest quality of anhydrous NaOAc available
 - (e.g., Thermo Scientific Dionex sodium acetate P/N 059326)
- Add the appropriate amount of 50% NaOH
 - (e.g., 5.2 mL for 1 L of 100 mM)
- Filter through a 0.2 μm nylon filter
- Store under helium. Prepare new eluent if >1 week old.
- Note: NaOAc eluents prepared without NaOH will have a short half-life (<1 week). If helium headspace is lost, prepare new eluent.

Starting with Acetate in the Eluent



Column: Thermo Scientific Dionex CarboPac™ PA100 + guard

Eluent: A) 0–30 min, 100 mM Sodium hydroxide/
0–210 mM sodium acetate;
30–31 min, 100 mM sodium hydroxide/
210–0 mM sodium acetate;
31–40 min, 100 mM sodium hydroxide/
0 mM sodium acetate

B) 0–30 min, 100 mM Sodium hydroxide/
24–210 mM sodium acetate;
30–31 min, 100 mM sodium hydroxide/
210–24 mM sodium acetate;
31–40 min, 100 mM sodium hydroxide/
24 mM sodium acetate

Flow Rate: 1.0 mL/min

Inj. Volume: 10 μ L

Temperature: 30 °C

Detection: PAD waveform B (TN 21)

Sample: Water

Postcolumn NaOH for HPAE-PAD

- Use for separations not performed at high pH or those performed at < 5 mM NaOH that require more sensitivity
- Use a tee to add 0.3 M NaOH at 0.5 mL/min
- Add by pressurizing, using a Thermo Scientific Dionex PC10 postcolumn delivery device
- Measure the combined flow rate to determine if 0.5 mL/min of the postcolumn NaOH has been achieved
- Monitor pH of combined mobile phases

Shutting Down an HPAE-PAD System

- For short term (a few days), turn off the cell, stop flow or flow a strong eluent (100 mM NaOH or stronger) at low flow rate.
- For long term, fill column with strong eluent, remove from the system, and cap.
- Remove reference electrode and put it in its container with a 3 M KCl solution.*
- Pump water through the system (each channel used). This can be done at 5 mL/min when no column is in line. Flush the autosampler.

* See the Electrochemical Detector Manual for more detail.

Which Thermo Scientific Dionex CarboPac Column Should I Use?

Application	Column
Monsaccharide Analysis—Glycoproteins Monosaccharide Analysis—Plants Monosaccharide Analysis—Rhm-GalN Required Monosaccharide Analysis—Acetylated and Neutral Sugars Mono-, Di-, and Trisaccharides	Dionex CarboPac™ PA20, PA10, or PA1 Dionex CarboPac PA1 (rev. grad.) Dionex CarboPac PA1 Dionex CarboPac MA1 Dionex CarboPac SA20, PA20, PA10, or PA1
Oligosaccharide Analysis Methylated Galacturonic Acids	Dionex CarboPac PA100, PA200 Dionex CarboPac PA1*
Sugar Alcohols Regulated methods (e.g. AOAC 996.04 – Sugars in Molasses)	Dionex CarboPac MA1 Dionex CarboPac PA1**
Sialic Acids	Dionex CarboPac SA20, PA10 (PA1) (PA20)

* From a literature reference.

** All current regulated methods specify the PA1.

Cleaning Thermo Scientific Dionex CarboPac™ Columns

- Always disconnect the column from the amperometry cell
- Remember that replacing a guard column may be more cost effective
- First try ten column volumes of strong eluent (e.g., 1 M NaOH or 1 M NaOAc [except Dionex CarboPac MA1])

Aggressive Cleaning for Thermo Scientific Dionex CarboPac™ PA1, 10, 20, and 1(2)00

- Clean the column with 60 mL of 1.0 M HCl or methanesulfonic acid
- Then clean the column for 1.5 hours with 200 mM NaOH
- Reconnect the cell, equilibrate the column under the desired initial conditions
- Test the column with standards (test chromatogram) to ensure that good column performance has been restored

Aggressive Cleaning for the Thermo Scientific Dionex CarboPac™ MA1

- Wash the column extensively (50 column volumes) with water. At 0.4 mL/min, this will require about 3.5 hours.
- Clean the column with 50 mL of 1.0 M of acetic acid
- Wash the column with 50 mL of water
- Clean the column with 50 mL of 1.0 M sodium acetate
- Wash the column with 50 mL of water
- Clean the column with 50 mL of 0.5 M sodium hydroxide
- Equilibrate the column under the desired initial conditions. Test the column with standards (test chromatogram) to ensure that good column performance has been restored.

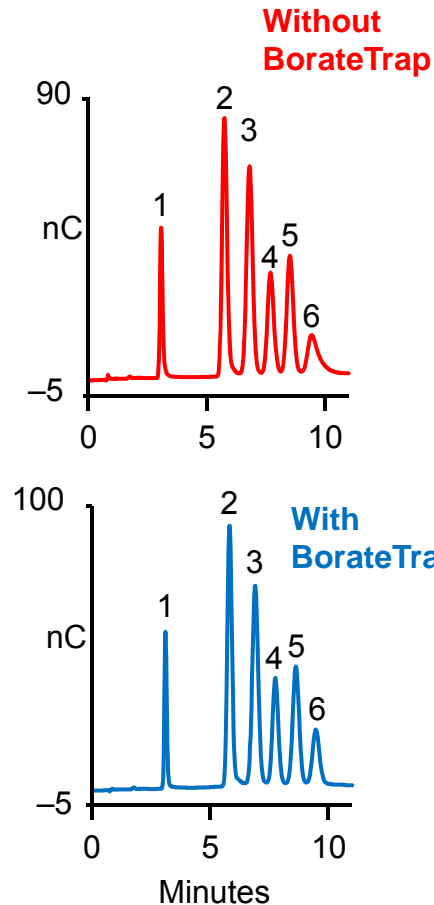
Other Columns Used with Carbohydrate Analysis

Column	Descriptions
Dionex Amino Trap™ Column	The Thermo Scientific Dionex AminoTrap column is used immediately before the Thermo Scientific Dionex CarboPac™ PA10 or PA20 columns and serves to delay the elution of the amino acids until after the monosaccharides have eluted. The gold electrode is therefore protected from contamination. The amino acids are eluted during the regeneration step, at high hydroxide concentration, when the electrode is less susceptible to fouling.
Dionex Borate Trap™ Column	The Thermo Scientific Dionex BorateTrap column is used to clean up contaminated water when borate is present in the water used to make eluents for carbohydrate analysis. This column is intended to be used as a trap column between the pump and the injection valve for removing borate from hydroxide eluents.

Performance Enhancing Columns— Dionex BorateTrap

Effect of Borate and the Thermo Scientific Dionex BorateTrap™ Column on Mannose Peak Symmetry

- Borate affects monosaccharide peak symmetry, even when present at low ppb concentration range
- Borate is one of the first ions to break through a DI H₂O system
- Presence in eluent water causes a significant loss of peak efficiency, especially for mannose and reduced monosaccharides
- The Dionex BorateTrap column is used before the injection valve
- The Dionex BorateTrap column is not needed for systems with an eluent generator and CR-ATC



Column: Thermo Scientific Dionex CarboPac™ PA10
Eluent: 18 mM NaOH, 10 ng/mL borate
Flow Rate: 1.5 mL/min
Detector: Pulsed amperometry, gold electrode

Peaks: 1. Fucose
2. Galactosamine
3. Glucosamine
4. Galactose
5. Glucose
6. Mannose

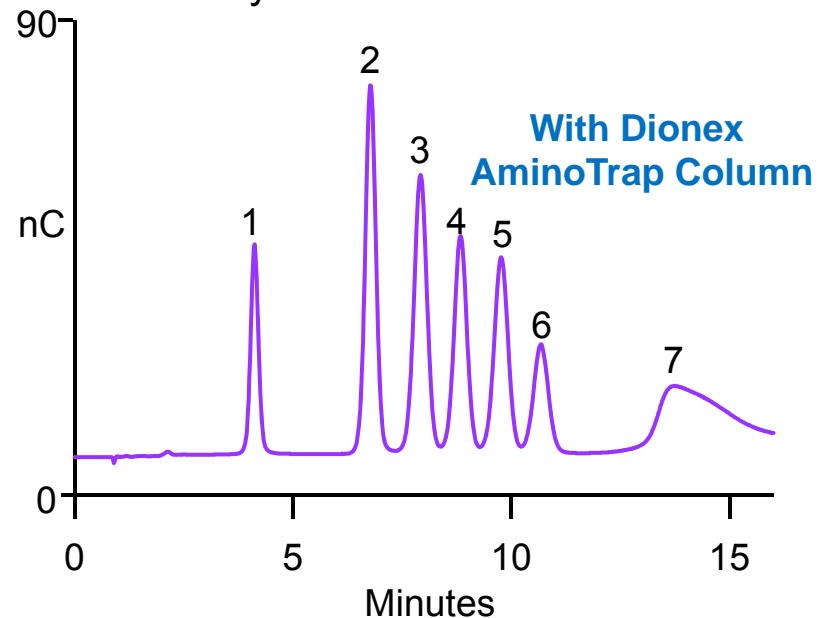
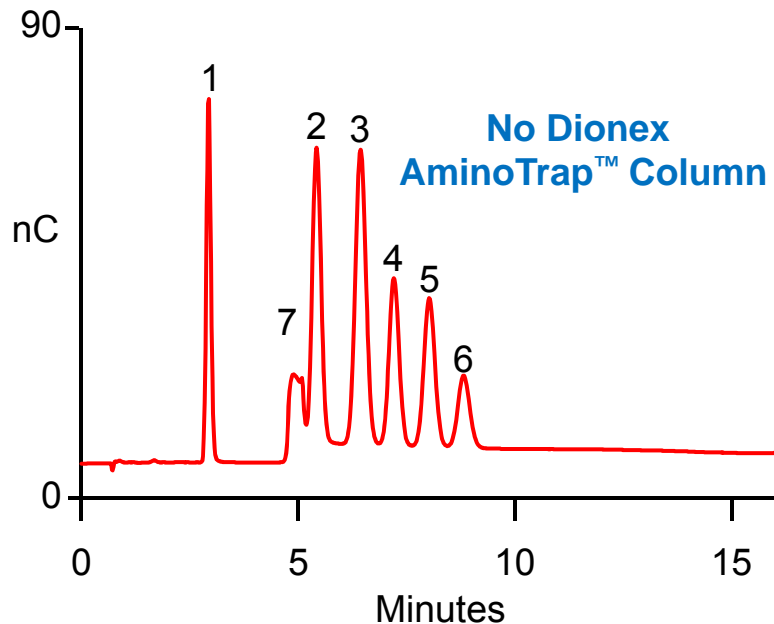
Performance Enhancing Columns— Dionex AminoTrap

- Monosaccharide detection is compromised by amino acids at the working electrode
- Apparent with amine-containing glycoconjugates with low levels of glycosylation
- Lysine elutes before galactosamine when the Thermo Scientific Dionex AminoTrap™ column is not employed, tails on the gold electrode
- Slow release of lysine's oxidation products inhibits detector response for later eluting monosaccharides
- The Dionex AminoTrap column resolves the quantitation problem by retaining lysine until after the monosaccharide's have been eluted
- If running NaOAc, AminoTrap column is not needed

Dionex AminoTrap Column Eliminates Lysine Interference

Column: Thermo Scientific Dionex
CarboPac™ PA10
Eluent: 18 mM Sodium hydroxide
Flow Rate: 1.5 mL/min
Detection: Pulsed amperometry,
gold electrode

Peaks: 1. Fucose 1 nmol
2. Galactosamine 1
3. Glucosamine 1
4. Galactose 1
5. Glucose 1
6. Mannose 1
7. Lysine 100



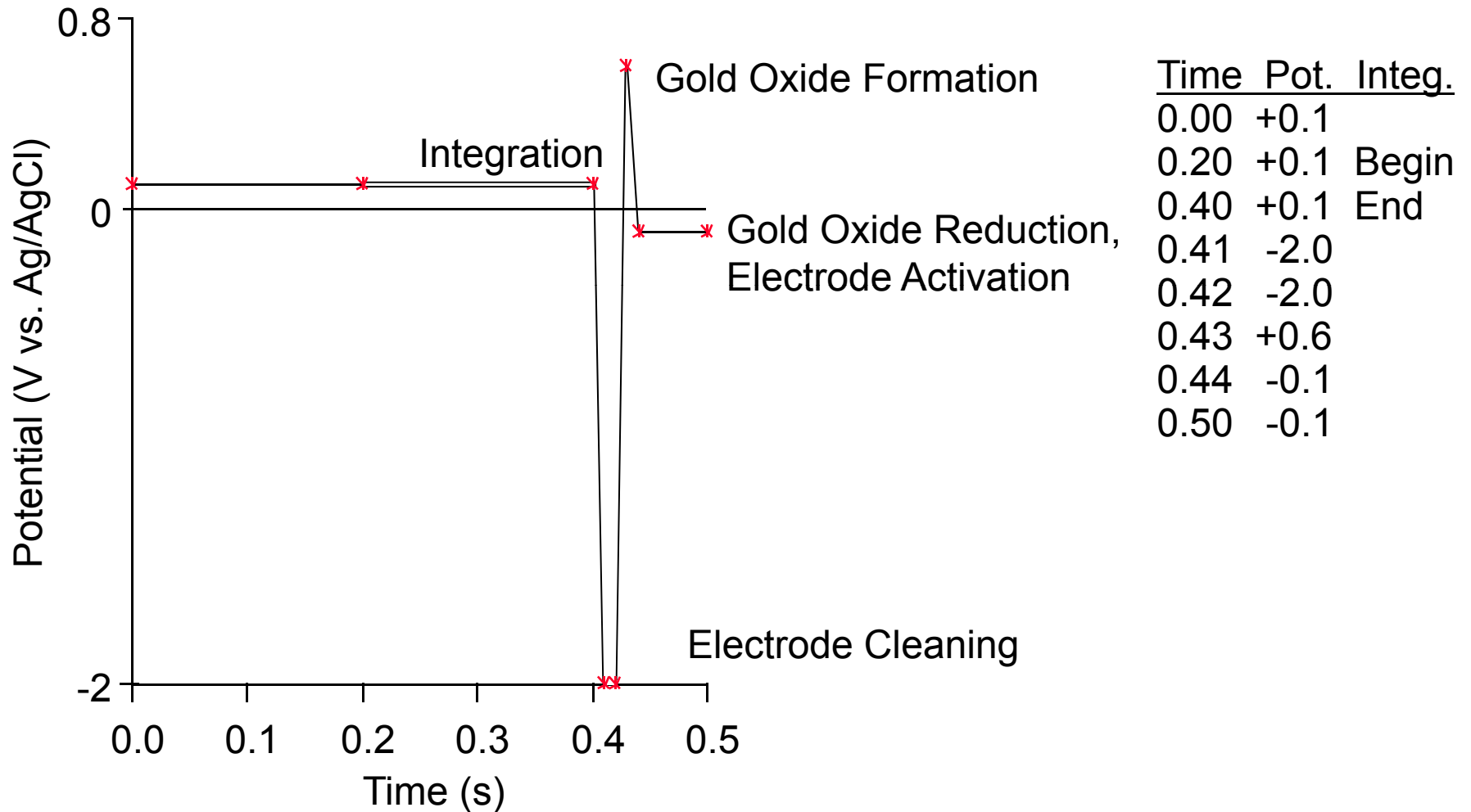
Functions of the Three Electrodes of the ED Cell Design

- Working Electrode—analyte is oxidized (or reduced), current through this electrode is measured
- Reference Electrode—sets the potential of the working electrode
- Counter Electrode—prevents current generated at the working electrode from passing through the reference electrode, which would alter the reference

Quadruple Potential Waveform A

- Dionex recommends using the quadruple waveform in order to take advantage of its long-term detection stability
- The waveform is pre-programmed into the ED50 and ED detector
- A more extensive discussion of waveforms for carbohydrate detection can be found in Dionex Technical Note 21, *Optimal Settings for Pulsed Electrochemical Detection of Carbohydrates*

Four-Potential Waveform (Waveform A, TN 21) for Carbohydrates



Thermo Scientific Dionex ICS-5000 ED Reference Electrode Calibration

Reference Electrode Calibration Procedure

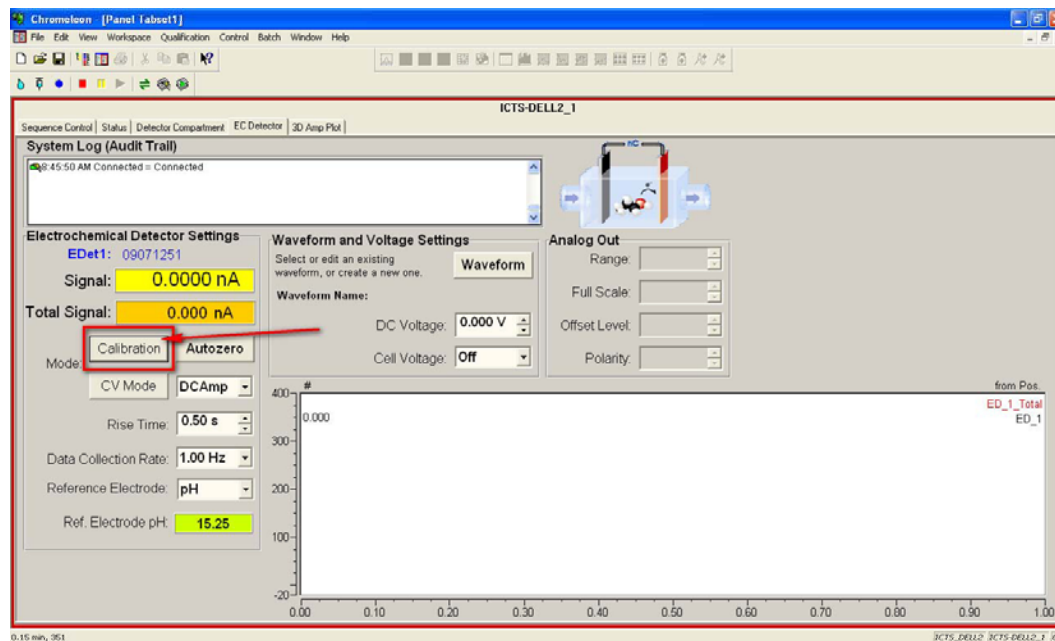
1. Calibrate after installing a new reference electrode

Items Needed

- A buffer solution with a pH of 7.00
- A second buffer solution with a different pH (typically one that matches the pH of the eluent used in your application, either pH 10 or pH 4 in most cases)

Thermo Scientific Dionex ICS-5000 ED Reference Electrode Calibration (Continued)

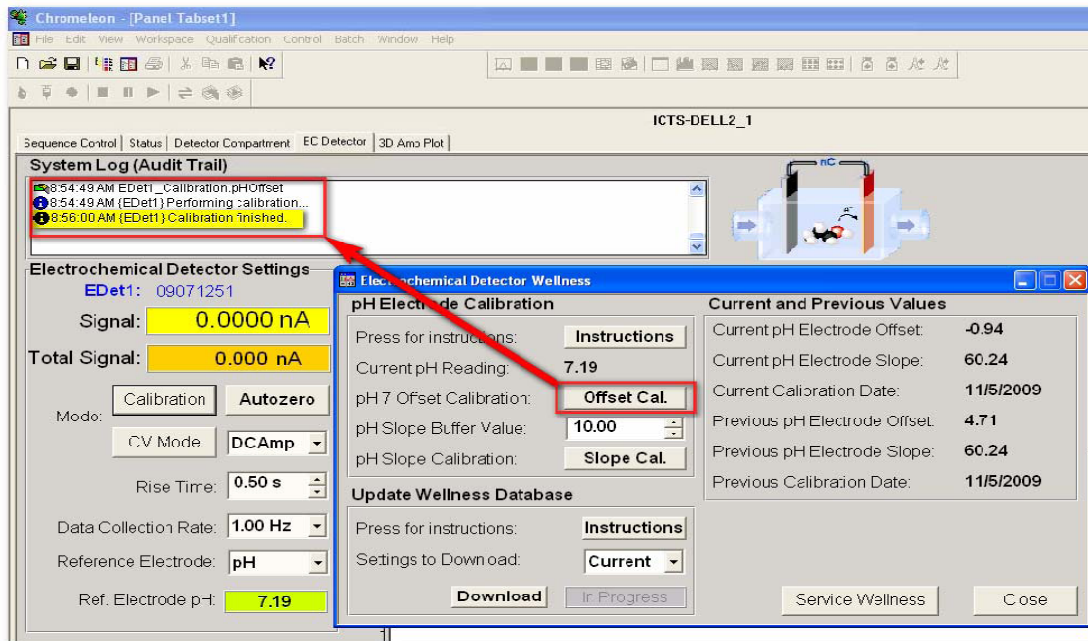
1. With the electrode removed from the cell, connect the electrical cables to the cell and electrode.
2. Open the Wellness panel in the Thermo Scientific Dionex Chromeleon™ or CMX software.



Reference Electrode
Calibration Procedure:
Click on Calibration Button

Thermo Scientific Dionex ICS-5000 ED Reference Electrode Calibration (Continued)

4. Place the electrode in the pH 7.0 buffer. Allow the pH to stabilize (about 1 minute), and then click the pH 7 button.



Reference Electrode
Calibration Procedure:
pH Offset Calibration at pH 7

5. Remove the electrode from the first buffer, rinse, and then dry it. Place the electrode in the second buffer solution. Allow the pH to stabilize.

ICS-5000 ED Ref Electrode Calibration

6. On the Wellness panel, enter the pH of the second buffer and then click the 2nd buffer button.

Chromeleon - [Panel Tabset1]

File Edit View Workspace Qualification Control Batch Window Help

ICTS-DELL2_1

Sequence Control | Status | Detector Compartment | EC Detector | 3D Amp Plot

System Log (Audit Trail)

- 11/14/09 AM (EDet1) ED pH slope calibration failed. Reference electrode needs to be recalibrated or replaced.
- 11/14/09 AM (EDet1) Error cleared: ED pH slope calibration failed. Reference electrode needs to be recalibrated or replaced.

Electrochemical Detector Settings

EDet1: 07120551

Signal: 0.0000 nA

Total Signal: 0.000 nA

Mode: Calibration Autozero

CV Mode DCamp

Rise Time: 0.50 s

Data Collection Rate: 1.00 Hz

Reference Electrode: pH

Ref. Electrode pH: 10.06

Electrochemical Detector Wellness

pH Electrode Calibration

Press for instructions: Instructions

Current pH Reading: 10.06

pH 7 Offset Calibration: Offset Cal.

pH Slope Buffer Value: 10.00

pH Slope Calibration: **Slope Cal.**

Update Wellness Database

Press for instructions: Instructions

Settings to Download: Current

Download In Progress

Service Wellness Close

Current and Previous Values

Current pH Electrode Offset:	1.88
Current pH Electrode Slope:	60.93
Current Calibration Date:	11/11/2009
Previous pH Electrode Offset:	2.83
Previous pH Electrode Slope:	60.93
Previous Calibration Date:	11/11/2009

Reference Electrode
Calibration Procedure:
pH Slope Calibration
at pH 10

Replacing Amperometry Cell Reference Electrode

When to Replace the Reference Electrode

- Replace the reference electrode if performance problems occur that are not corrected by regenerating the electrode

To regenerate a reference electrode, soak it in a solution of 1 M KCl and 1 M HCl

- Performance problems can include:
 - No pH readouts
 - Shift in Ag/AgCl reference potential or incorrect readouts
 - Baseline spikes
 - Decreased response even with a freshly polished working electrode
- The reference electrode typically lasts about six months (Thermo Scientific Dionex ICS-3000/5000) or three months (Thermo Scientific Dionex ED40/50/50A) in normal use

Replacing Amperometry Cell Ref Electrode



IMPORTANT



Always store the electrode in the storage bottle filled with saturated KCl solution when the cell is not in use. This prevents the reference electrode membrane from drying out and damaging the electrode.

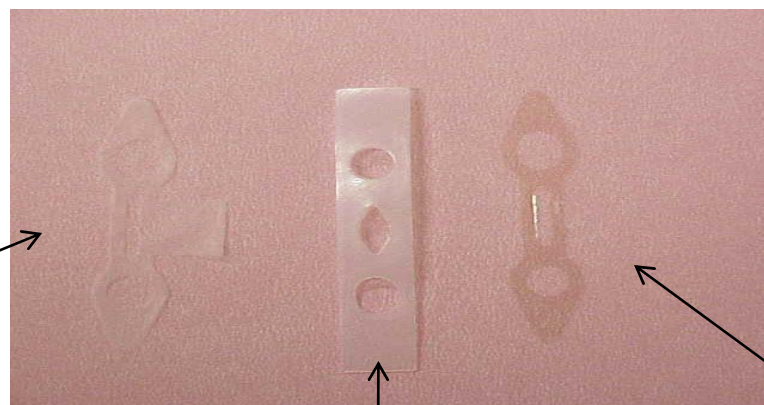
- Rinse the electrode thoroughly in ASTM filtered, Type I (18 M Ω -cm) DI H₂O to remove any precipitated salt
- Calibrate the reference electrode, check that the reference electrode and storage cap O-rings are in place, then reassemble the cell

Amperometry Cell Gaskets

When to Replace the Gasket

Replace the gasket if there is a leak between the gasket and electrode, or between the gasket and cell body

Three Different Types of Gaskets in Use with ED40/50



Use with disposable electrodes
Material: PTFE
Thickness: 2 mil
PN 060141

Used to expand range of
linearity with non-disposable
electrodes
Material: ultrahigh-density
polyethylene (UHDP)
Thickness: 15 mil
PN 057364

Standard gasket for standard
non-disposable electrodes
Material: Ultem
Thickness: 1 mil
PN 045972

All three ED50 gasket types can be
used with ED3000/5000

Electrodes and Applications

Disposable Electrode	Conventional Electrode	Applications	Example Columns	Typical Conditions		Additional Comments
				Hydroxide	MSA	
Gold Carbohydrates : 060139, 060216 (on polyester) AAA-Certified: 060082, 060140 (on polyester)	Gold ED40/50 Carbohydrates: 044108 AAA: 055832	Carbohydrates	CarboPac PA20	Up to ca. 1 M	N/A	Use polyester-based DE only up to 100 mM hydroxide** For higher hydroxide concentrations, use Gold on PTFE (see below) or conventional Gold electrodes.
	Gold ED (ICS- 3000) Carbohydrates: 061749 AAA: 063722	AAA-Direct	AminoPac PA10			
Gold on PTFE: 065480		Carbohydrates and sugar alcohols [#]	CarboPac MA1	Up to 750 mM	N/A	4 week life time; no NaOH limit (tested to 750 mM)
Silver: 063003	Silver ED40/50 044114 Silver ED (ICS- 3000) 061755	Cyanide, Sulfide, Bromide, Iodide	IonPac AS7	62.5 mM	N/A	For higher hydroxide concentrations (above 100 mM), use conventional Silver electrodes.
Platinum: 064440	Platinum ED40/50 044113 Platinum ED (ICS-3000) 061751	Alcohols and Chelating agents	IonPac ICE-AS1	N/A	100 mM	For higher MSA concentrations (above 100 mM) use conventional Platinum electrodes.
	3 mm Gold ED (ICS-3000)	Aminoglycosides	Polymeric RP, Silica RP	0.5 M NaOH added post column	N/A	Post-column addition of hydroxide
Carbon on PEEK: 069336		S-containing amino acids, electroactive nucleosides, catecholamines	OmniPac PCX-500	N/A***	N/A***	

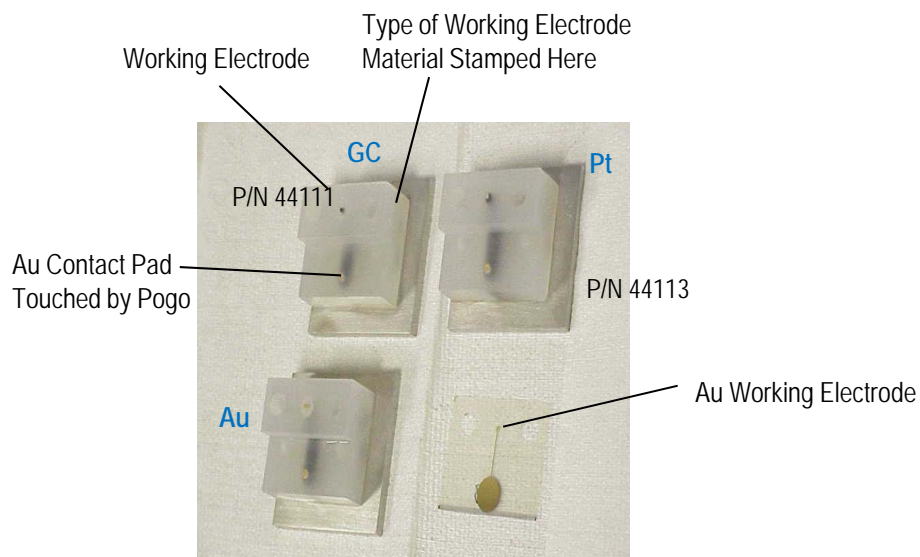
* For a detailed description of all applications of electrochemical detection, see the Product Manual for Disposable Electrodes (Doc. No.: 065040).

** With continuous exposure. For short (5–10 min) periods of time, up to 200 mM NaOH may be used.

*** 0.1 M Phosphate buffer (pH = 2) with 10% Methanol.

Not to be used with AAA-Direct.

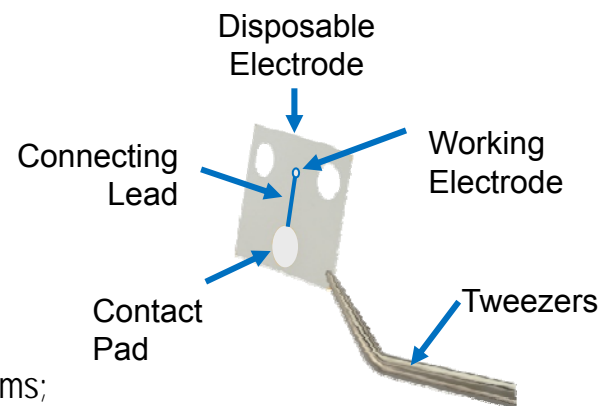
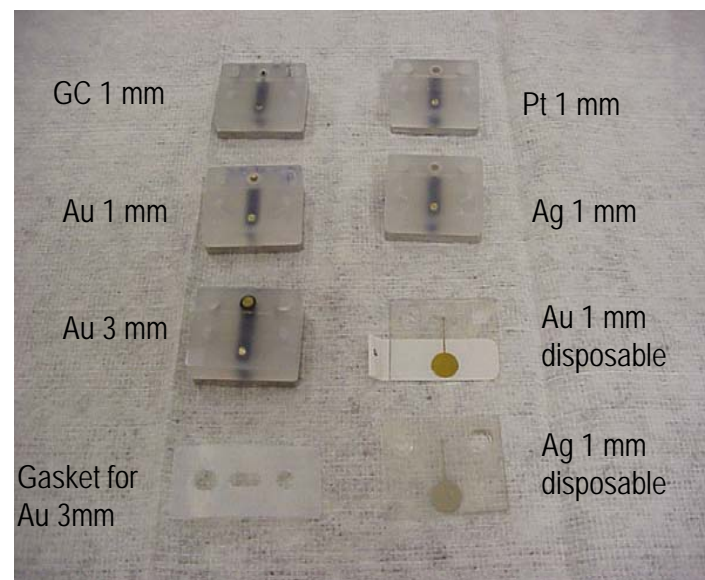
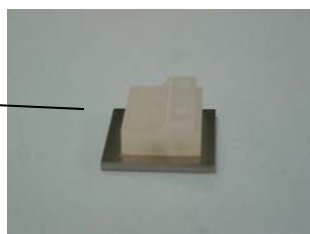
Conventional and Disposable Electrodes



P/N 55832,
P/N 44112
not shown:
P/N44114
"Ag"

Disposable Electrode
for ED50: P/N 60082 and
60139

Blank Block for
Use with DWE



ED50 working electrodes can not be used with Dionex ICS-3000/5000 systems;
exception: disposable electrodes. All three ED50 gasket types can be used with Dionex ICS-3000/5000 systems.

Why Disposable Electrodes?

- Efficient and reproducible manufacturing
- Cost-effective in QC and high-throughput environment
- Easy installation
 - Fast equilibration (minimal delay after installation)
 - Excellent electrode-to-electrode reproducibility
- Predictable stability of response
- No conditioning required
 - No hand polishing
 - No activation waveforms

References:

1. J. Cheng, P. Jandik and N. Avdalovic, *Anal. Chem.* **2003**, 75, 572–579.
2. J. Cheng, P. Jandik and N. Avdalovic, *Anal. Chim. Acta* **2005**, 536, 267–274.

Gold Disposable Working Electrode

- Do not exceed 100 mM hydroxide concentration when using polyester (PE)-based disposable electrodes
- However, 2–5 minute-long rinsing intervals with 200–500 mM hydroxide can be tolerated within 20–40 minute eluent programs
- PTFE-based disposable electrodes are rated up to 750 mM NaOH, so this might be a good alternative to PE DWEs
- There are no limitations on the concentration of sodium acetate within the recommended range for Thermo Scientific Dionex CarboPac™ columns (0–1.0 M)

Disposable Au Working Electrodes and Background

- With disposable Au working electrodes we observe different backgrounds than observed with conventional electrodes.
- Our current data suggest that a properly functioning system has backgrounds of:
 - 15–30 nC for 10 mM hydroxide
 - 35–60 nC for 10 mM hydroxide, EG system
 - 20–40 nC for 100 mM hydroxide, 100–500 mM acetate

Conventional Au Working Electrode

- For long term stability use the quadruple waveform
- Recession (not flat with the polymer surface) replace (usually 6–8 months)
- For additional clean up procedure, refer to ED Manual

DO NOT TOUCH THE ELECTRODE SURFACE

Fat and other products come from your hand

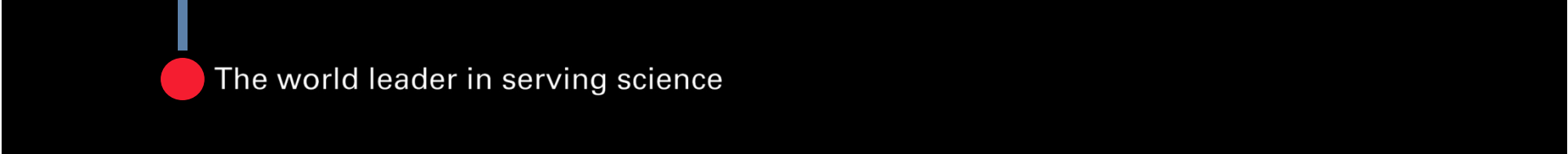
Recommended Reading Materials

1. USER'S COMPENDIUM for Electrochemical Detection, Document No. 065340, Rev. 1, 2010
2. Product Manual for Dionex CarboPac™ MA1, CarboPac PA1, CarboPac PA10, CarboPac PA100, Document No. 031824, Rev. 7, 2009
3. Product Manual for Dionex AminoPac™ PA10 *AAA-Direct*, Document No. 031481, Rev. 12, 2006
4. Product Manual for Disposable Gold Electrodes, Disposable Silver Electrodes, Disposable Platinum Electrodes, Disposable Carbon Electrodes, Document No. 065040, Rev. 7, 2009
5. Product Manual for Polishing Amperometry Cell Gold Working Electrodes, Document No. 031154, Rev.2, 2009
6. 3D Amperometry Training Manual Dionex ICS-3000 (or ICS-5000), Document No. TBD, Rev. 1, 2010

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● Thank You!



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Combination pH-Ag/AgCl Ref Electrodes

- pH calibration eliminates distortions of reference potential by the drift of Ag/AgCl potential

Note: only in pH referencing mode, not in Ag referencing mode

- Eliminates influence of pH on the detection signal with gold electrodes during gradient elutions

Note: only in pH referencing mode, not in Ag referencing mode

- pH read out used for monitoring of Ag/AgCl drift by following gradual changes over time in pH value during isocratic sections of chromatograms

($\Delta\text{pH } 0.5$ corresponds to ~ 30 mV change of Ag/AgCl potential)

Note: useful with Ag and pH referencing modes

- The combination electrode can be used as a separate detection channel