Thermo Scientific

Electrochemical Detection

User's Compendium

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Thermo SCIENTIFIC

User's Compendium

for

Electrochemical Detection (ED)

Revision History:

Revision 02, April, 2013; Rebranded for Thermo Scientific.

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

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

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1. Introduction

This Electrochemical Detection (ED) User's Compendium is designed to give users quick and easy access to relevant information for the following:

Electrochemical Detectors	• *ED (ICS-3/4/5000)* • *ED40* • *ED50/ED50A*
Electrochemical Detection Systems	• *Setup* • *Maintenance* • *Troubleshooting* • *Related Part Numbers* • *Legacy Systems*
Electrodes	• *Conventional Working Electrodes* • *Disposable Working Electrodes* • *Reference Electrodes*

This guide is written for both analytical and capillary ICS-5000 and ICS-5000$^+$ ED systems, the calippary-only ICS-4000 systems, the analytical-only ICS-3000 systems, as well as the older ED40, ED50/ED50A generation of detectors.

NOTE

Both Dionex and Thermo Fisher Scientific part numbers are used throughout this document. All Dionex part numbers are listed as "P/N XXXXXX" (6 digit numbers only) while Fisher part numbers are listed as "P/N XX-XXX-XXX" (three groups of numbers or letters separated by dashes).

1.1 References

This compendium is intended to be used in addition to the following Dionex (now part of Thermo Fisher Scientific) product manuals:

- Disposable Electrodes Manual (Doc. No.: 065040)
- Electrode Polishing Manual (Doc. No.: 031154)
- ICS-5000$^+$ Operators Manual (Doc. No.: 065446)
- ICS-5000 Operators Manual (Doc. No.: 065342)
- ICS-4000 Operators Manual (Doc. No.: 065468)
- ICS-3000 Operators Manual (Doc. No.: 065031)
- ED50 Operator's Manual (Doc. No.: 031673)
- ED50A Operator's Manual (Doc. No.: 031772)
- AAA-Direct Operator's Manual (Doc. No.: 031481)

NOTE

If the information required cannot be found in this compendium, please consult the operator's manuals mentioned above.

This compendium will serve as a primary reference source for basic troubleshooting, product information (including part numbers) and operator's manual references.

1.2 System Requirements

Thermo Fisher Scientific (formerly Dionex) electrodes are only compatible with Dionex-branded electrochemical detectors, models ED (ICS-3/4/5000), ED40 and ED50/ED50A. These electrodes cannot be installed on any other electrochemical detector. **Table 1** lists the recommended system components.

Table 1 System Component Recommendations

Module	ICS-3000, -4000 -5000 and -5000+Systems	Older DX and ICS System
Pump	SP/DP Gradient Pump or isocratic pump	GS50 or GP50 pump
Sample Delivery	Manual injector or AS-AP/AS autosampler	Manual injector or AS50 autosampler
Oven	DC, detector compartment	LC25, LC30 AS50TC column oven
Detector	ED	ED40, ED50, or ED50A
Column	Suitable Thermo Fisher Scientific Dionex brand Column	Suitable Thermo Fisher Scientific Dionex brand Column

1.3 System Operation Requirements

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

A. Eluent components kept under helium or high-purity nitrogen at all times.
B. On-line degassing of eluents.
C. Accurate and precise flow rates at 0.25 mL/min to 2 mL/min for 2 and 4 mm i.d. columns and 5 – 10 µL/min for capillary columns (depending on the application).
D. pH/Ag/AgCl and Ag/AgCl* (analytical or capillary flow rates) or PdH (capillary only) reference electrode.
E. Programmable Pulsed or Integrated Amperometry waveforms with frequencies of 0.50 Hz or higher.
F. Electrochemical-grade chemicals (sodium acetate, sodium hydroxide) and high purity deionized ASTM Type I (18.0 MegOhm-cm or better) water (not HPLC water) to achieve low detector background levels.
G. Column oven, for constant temperature control of the guard column, separation column, and detection cell.
H. The heat exchange coil in the AS50 thermal compartment must be 0.005” (0.125 mm) inner diameter (ID), 1/16”
outer diameter (OD) PEEK tubing (P/N 052311) for 2 mm ID column applications (this does not apply to AS or AS-AP autosamplers).
I. All tubing between the injector and detector cell inlet must be ≤0.005” (0.125 mm) ID with 2 mm ID (microbore) columns.

* pH/Ag/AgCl and Ag/AgCl are simplified to be “pH” and “AgCl”, respectively, in Chromeleon software.

1.3.1 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 to protect the ion exchange columns and system components. Thermo Fisher Scientific cannot guarantee performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

1.3.1.1 Deionized (DI) Water

The DI water used to prepare eluents should be ASTM Type I reagent grade water with a specific resistance of 18 MegOhm-cm, or better. The water should be free of ionic 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 may lead to high background signals and gradient artifacts. If water quality is an issue, Thermo Fisher Scientific recommends the Barnstead Nanopure water purification system, Thermo Fisher Scientific P/N D11971.

Table 2 Purity Requirements for Chemicals

Chemical	Part Numbers	Notes
Sodium Hydroxide	033465 or SS-254-500	50% w/w solution
Sodium Acetate	059326	Thermo Fisher Scientific cannot guarantee proper detection performance when different grades or alternate suppliers are used.
Methanesulfonic Acid (> 99%)	033478	

1.4 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 ASTM Type I, 18.0 MegOhm-cm water (or better) at the installation site.
- ☐ Vacuum pump available for use with the vacuum filtration units.
- ☐ Inert gas cylinder (helium or nitrogen) with a pressure 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 pipettes 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 pipettes for transferring MSA.
- ☐ Dedicated vacuum filtration unit (Thermo Fisher Scientific P/N 09-740-46).

1.4.1 The Most Important Rules

Always…

A. Use only recommended reagents for preparation of eluents.

B. Use dedicated glassware and disposable glass or plastic ware for volume adjustments.

C. Keep 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 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.

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 above 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 and replace 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 (ICS-3000 system) or 5,000 psi (ICS-4000 and -5000 systems).

1.5 Care and Use of Electrodes

1.5.1 Conventional Working Electrodes

Before the introduction of disposable electrodes by Thermo Fisher Scientific, Conventional Working Electrodes (CWE) were the standard used in electrochemical detection. These electrodes offer consistent results with very good lifetimes but sometimes require longer equilibration times once newly installed in a system. Surface re-polishing of CWEs when performance degradation is observed, including electrode fouling after system contamination, can be performed to regain performance in some cases. CWEs can offer excellent lifetimes if operated under recommended conditions and stored properly (in the shipping case) when not in use.

1.5.2 Disposable Working Electrodes

Disposable Working Electrodes (DWE) are the latest innovation in electrochemical detection. They offer a cost effective alternative to CWEs in many laboratories, especially in QC and high-throughput environments. Disposable electrodes offer consistent, stable responses with fast equilibration times and excellent electrode-to-electrode reproducibility. While disposable electrodes do not require activation waveforms or polishing, a finite lifetime before replacement is specified (approximately 1 week for gold on polyester (AAA), 2 weeks for gold on polyester (carbohydrate), 4 weeks for gold on PTFE, 2 weeks for disposable platinum, silver and carbon disposables).

1.5.3 Reference Electrodes

1.5.3.1 pH/Ag/AgCl Reference Electrode

pH/Ag/AgCl reference electrodes can be operated for 3-6 months (depending on the application) from the first date of use. The glass membrane of the reference electrode must always remain in solution and never allowed to dry out. It is recommended that the reference electrode be removed from the system, should the system remain unused for extended periods of time (i.e., for more than 2 days). Long-term storage of the reference electrode (if not being used) should be in a saturated potassium chloride solution, and not in the instrument itself.

1.5.3.2 Palladium Hydrogen Reference Electrode

With the introduction of capillary ion chromatography on the ICS-4000 and -5000 platform, a new reference electrode was introduced. This solid state reference electrode has an extended lifetime compared to Ag/AgCl reference electrodes, but does not offer the option of reporting a pH value. This reference electrode does not need to be calibrated in software but does need to be charged before use (to establish a thin-layer of palladium hydride at the electrode surface) the procedure is in Section 2.5 later in this document. Storage of the PdH RE does not require any storage solutions. **However, be sure to rinse the sensing surface with water thoroughly and dry it under a stream of nitrogen. Use the red cap shipped with the electrode if you store the electrode for a longer time (>1 hour).**

1.5.4 Testing of Gold Working Electrodes

Three procedures are in wide-spread use for determining the status of gold working electrodes; glucose, galactosamine and histidine testing. All three tests will show the current status of the working electrode which is very useful as a troubleshooting procedure.

1.5.4.1 Glucose Test – Analytical System

- Inject 25 µL of an 8 µM glucose standard solution using a CarboPac PA1 (4 x 250 mm) column (P/N 035391) and 100 mM sodium hydroxide eluent at a flow rate of 1.0 mL/min.
- An 8 µM glucose standard can be prepared from a 1 mM stock solution made by dissolving 18 mg of glucose into 100 mL of DI water.
- The detection is performed with the Quadruple Potential Waveform, using a 1.0 mil Ultem gasket (P/N 045972) with conventional electrodes or a 2 mil Teflon gasket (P/N 060141) with the disposable electrodes.
- The peak area counts for this injection should be $\geq$6.3 nC*min.

If this test fails, then the system may be contaminated or the electrode needs to be replaced. Should this test fail after a system clean-up has been performed, a conventional working electrode should be polished, and if using a disposable electrode, it should be replaced. Low area counts in this test may also be the result of a Ag/AgCl reference electrode with a potential differing by more than 30 mV from the theoretical value.

1.5.4.2 Glucose Test – Capillary System

- Inject 0.4 µL of an 10 µM glucose standard solution using a CarboPac PA20 (0.4 x 150 mm) column (P/N 072117) and 10 mM KOH eluent at a flow rate of 10 µL/min.
- A 10 µM glucose standard can be prepared from a 1 mM stock solution made by dissolving 18 mg of glucose into 100 mL of DI water.
- The detection is performed with the Quadruple Potential Waveform, using a 1.0 mil Ultem gasket (P/N 045972) with a conventional electrode. The detection can also be carried out with a disposable gold electrode and a 1.0 mil PTFE gasket (P/N 072117).
- The peak area counts for this injection should be $\geq$2.0 nC*min.

If this test fails, then the system may be contaminated or the electrode needs to be replaced. Should this test fail after a system clean-up has been performed, a conventional working electrode should be polished, and if using a disposable electrode, it should be replaced. Low area counts in this test may also be the result of a Ag/AgCl reference electrode with a potential differing by more than 30 mV from the theoretical value.

1.5.4.3 Galactosamine Test – Analytical System

This test is routinely performed on new Disposable Electrodes and the results are documented in the Quality Assurance Record (QAR) sheet shipped with each packet of electrodes. Inject 10 µL of 10 µM galactosamine solution using a CarboPac PA20 (3 x 150 mm) column (P/N 060142) and 10 mM sodium hydroxide eluent at a flow rate of 0.5 mL/min.

- The detection is performed with the Quadruple Potential Waveform, using a 1.0 mil Ultem gasket (P/N 045972) with conventional electrodes or a 2 mil Teflon gasket (P/N 060141) with disposable electrodes.
- Peak area counts for this test should be ≥6.0 nC*min.

If this test fails, then the system may be contaminated or the electrode needs to be replaced. Should this test fail after a system clean-up has been performed (Refer it to Sections 1.6 ad 1.7), a conventional working electrode should be polished, and if using a disposable electrode, it should be replaced. Low area counts in this test may also be the result of a Ag/AgCl reference electrode with a potential differing by more than 30 mV from the theoretical value.

1.5.4.4 Histidine Test – Analytical System (AAA Application)

- Inject 25 µL of 8 µM histidine standard solution using an AminoPac PA10 Guard (2 x 250 mm) column (P/N 055406) and 62.5 mM NaOH / 400 mM NaAc eluent (36% water / 24% 250 mM NaOH / 40% 1 M NaAc) at a flow rate of 0.25 mL/min.
- A 8 µM histidine standard solution can be prepared from a 1 mM stock solution made by dissolving 155 mg of histidine (Sigma-Aldrich, P/N: H7750, MW 155.15) into 100 mL of DI water (A 20 ppm sodium azide aqueous solution is preferred to be used instead of Di water as the diluent if available.).
- The detection is performed with the AAA Waveform, using a 1.0 mil Ultem gasket (P/N 045972) with conventional electrodes or a 2 mil Teflon gasket (P/N 060141) with disposable AAA Au electrodes.
- Peak height counts for this test should be ≥200 nC for conventional AAA Au working electrodes or > 280 nC for disposable AAA Au working electrodes.

If this test fails, then the system may be contaminated or the electrode needs to be replaced. Should this test fail after a system clean-up has been performed, a conventional AAA Au working electrode should be polished, and if using a disposable AAA Au electrode, it should be replaced. Low area counts in this test may also be the result of a Ag/AgCl reference electrode with a potential differing by more than 30 mV from the theoretical value.

NOTE

The cleaning procedures in the following Sections 1.6 are meant for capillary systems. Similar procedures for use with analytical systems are described in Sections 1.7.

1.6 Cleaning Procedures for Capillary Systems with Electrochemical Detection

1.6.1 Cleaning Procedure with 2 M KOH for Reducing High Background and Low Response

The following procedure for the removal of possible bacterial contaminants details the recommended steps for ICS-4000 and capillary ICS-5000 systems equipped with an ED detector and capillary CarboPac PA20 column.

Steps to decontaminate a capillary system with 2 M KOH (**It has to be KOH, if NaOH is used, EG module has to be bypassed**) Molecular Weight of KOH: 56.11 g/mol

1. Turn off Eluent Generator (EG), turn off CR-ATC and stop the pump.
2. Remove the capillary CarboPac PA20 column set and ED cell from the system.
3. Restore the liquid connection between the injector valve and the tubing normally connected to the outlet of detection cell (columns and ED cell have been removed) with ca. 100-inch PEEK tubing (ID: 0.0025”). The cell outlet tubing remains connected to Suppressor Bypass.
4. Prepare 0.2-L of 2 M KOH (Preferably by dilution from KOH, 45% w/w, 11.63 M, P/N: 3143-01, J T Baker). Filter ~0.18 L DI water through a 0.2-µm Nylon membrane filter, add 34.4 mL of the 45% KOH, fill up to 0.20 L. Discard water from E1 eluent bottle and replace it by the filtered aliquot of 0.2-L of 2 M KOH (shake well after blanketing with inert gas).
5. Disconnect the CR-ATC outlet tubing from the inlet of EG degasser, pump at priming flow rate at least 30-mL of KOH into waste with the priming valve closed. The pump outlet tubing remains connected to the EG Cartridge (EGC) inlet , the 2 M KOH passes through the EGC and CR-ATC and through the tubing connected to CR-ATC outlet into waste (By doing so, we make sure that the pulse damper of the ICS-5000 DP and all tubing upstream of the degasser is being rinsed with 2 M KOH.) Please make sure that the drain tubing does not generate an excessive pressure. Otherwise, please lower the flow rate.
6. Stop the pump. Re-connect the CR-ATC outlet tubing to the degasser inlet. Pump at least 8-mL of 2M KOH (flow rate at least 0.03-mL/min, preferably at 0.10 mL/min if possible) from the eluent bottle through the system into detector outlet tubing that remains connected to Suppressor Bypass.
7. Remove the 100-inch PEEK tubing added in step 3, connect the injection valve to the CarboPac PA20 column set and connect the outlet from the CarboPac PA20 column to the tubing that is normally connected to the ED cell outlet. The ED cell remains bypassed.
8. Reduce the flow rate to 0.01-mL/min and continue rinsing with 2 M KOH for another hour.
9. Toward the end of the 2 M KOH rinse, turn the injection valve at least 3 times between inject and load positions for 30 seconds each time.
10. After finishing the rinse with 2 M KOH, replace the 2 M KOH solution with filtered ultrapure water blanketed with inert gas in the eluent bottle. Prime the line the same way as in step 5.
11. Rinse the system flow path with water at 0.010 mL/min for 20 minutes. Turn the injection valve as in step 9.

12. Stop the flow and install the ED cell into the system. Restart the pump (0.01 mL/min).
13. Turn on EG and CR-ATC. Select 100 mM KOH concentration and pump the KOH solution through the cell until the reference electrode indicates pH ~13.
14. Generate a chromatogram of 10 µM monosaccharide standard under the conditions of QAR for capillary CarboPac PA20 column. Note: Perform a full loop 0.4 µL injection (not the timed injection specified in the QAR).
15. The peak area of galactosamine should be >6 nC min.

1.6.2 Cleaning Procedure with 6.5 mM EDTA for Improving Response

The following procedure for the removal of possible heavy metal contaminants details the recommended steps for ICS-4000 and capillary ICS-5000, ICS-5000$^+$ systems equipped with an ED detector and capillary CarboPac PA20 column.

Steps to decontaminate a capillary system with 6.5 mM (2.418 g/L) Na_2EDTA (must be the disodium EDTA form) Molecular Weight: 372 g/mol

1. Turn off Eluent Generator (EG), turn off CR-ATC, stop the pump.
2. Disconnect the pump outlet tubing from the EG Cartridge (EGC) inlet.
3. Remove the capillary CarboPac PA20 columns from the system.
4. Remove the gold (Au) electrode from the cell, close the cell again using an empty holder block over a gasket.
5. .
6. Restore the liquid connection between the injector valve and detection cell (column has been removed) with a 100-inch PEEK tubing (ID: 0.0025”).
7. Prepare 0.2-L of 6.5 mM Na_2EDTA (must be the disodium form) and filter it through a 0.2-µm Nylon filter.
8. Discard water from E1 eluent bottle and replace it by the filtered aliquot of 6.5 mM EDTA.
9. Pump at priming flow rate at least 30-mL of EDTA into waste with the priming valve closed. The pump is drained out of the pump outlet tubing you disconnected from the EG C inlet in step 2. (By doing so, we make sure that the pulse damper of the ICS-5000 DP is being rinsed with EDTA.) Please make sure that the drain tubing does not generate an excessive pressure. Otherwise, please replace it with a short segment of black tubing (ID: 0.010”).
10. Stop the pump flow. Connect the pump outlet tubing to the tubing normally used between the outlet of CR-ATC and degasser inlet. Pump at least 10-mL of EDTA (flow rate at least 0.03-mL/min, preferably at 0.10 mL/min if possible) from the eluent bottle through the system into detector waste. The cell outlet tubing remains connected to the Suppressor Bypass.
11. Toward the end of the EDTA rinse, turn the injection valve at least 3 times between inject and load positions for 30 seconds each time.
12. After completing the rinse with EDTA, replace the 6.5 mM EDTA cleaning solution with filtered ultrapure water blanketed with inert gas in the eluent bottle. Prime the line the same way as in step 7.
13. Rinse the system flow path (column remains out of the system) with water as in step 7 but only for 10 minutes. Turn the injection valve as in step 9Re-connect EG and CR-ATC into the liquid path between the pump and EG degasser. Start the pump (10 µL/min).

14. Turn on the EG and CR-ATC. Select 100 mM KOH concentration and pump the KOH solution through the cell until the reference electrode indicates pH >12.
15. Stop flow and reinstall the CarboPac PA20 column set.
16. Install a new disposable electrode (reinstall a conventional gold electrode after polishing) and generate a chromatogram of 10 µM monosaccharide standard under the conditions of QAR for capillary CarboPac PA20 column. Note: Perform a full loop 0.4 µL injection (not the timed injection specified in the QAR).
17. With a new disposable gold electrode, the peak area of galactosamine should be >6 nC min.

1.6.3 System Cleaning Procedure with 2 M Sodium Hydroxide

Whenever microbial system fouling is suspected, the application of a strong base rinse (*e.g.*, 2 M NaOH) followed by copious flushing with DI water may be required. It is recommended to perform this procedure at the time of initial installation, after a hardware preventive maintenance cycle, when new PEEK tubing is replaced (if necessary), as an initial troubleshooting step (if a column test standard response indicates low response) or as part of a general system maintenance protocol.

This cleaning procedure with 2 M NaOH is for an ICS-3/5000 system equipped with a quaternary low pressure gradient option:

A. Prepare a solution of 2 M NaOH by diluting 104 mL of 50% w/w NaOH (P/N 033465) to 1 L with DI H_2O.
B. Manually rinse the eluent bottle with the prepared 2 M NaOH (50 mL or more) followed by a rinse with several hundred mL of DI H_2O.
C. Place the 2 M NaOH in the pre-rinsed bottle and place all 4 eluent lines (A,B,C,D) in it; if possible maintain under a stream of inert gas.
D. Withdraw at least 40 mL of NaOH from each line, using a syringe to ensure that each line is primed with the 2 M NaOH. Ensure that the detector and column are bypassed then proceed to Step (E).
E. Close the solvent draw-off valve and leave the pump proportioning at 25/25/25/25 for 15 mins. or longer.
F. Make sure all surfaces come into contact with the flush solution; rotate the injection valve.
G. Repeat steps (D)-(F)with 18.2 MegOhm-cm DI H_2O.

1.6.4 Steps to remove metallic contamination from the system with 6.5 mM (2.4 g/L) Na_2EDTA (must be the disodium EDTA form) Molecular Weight: 372 g/mol

Use this procedure for ICS3000/5000 systems with a low-pressure quartenary gradient pump (analytical channels)

A. Remove the column.

B. Remove the gold (Au) electrode from the cell, close the cell again using an empty holder block over a gasket.

C. Restore liquid connection between the injector valve and detection cell (column has been removed).

D. Empty the contents of eluent container E1, rinse it with 1 L of 18 MegOhm-cm water and discard the water.

E. Filter 1 L of 18 MegOhm-cm water through 0.2 µm Nylon filter. Note: Do not use any other material than Nylon for eluent filtration.

F. Transfer the filtered water into the eluent container E1. Pump at least 30 mL of water into the pump waste at priming flow rate.

G. Stop the pump flow. Close the priming valve. Pump at least 50 mL of water from E1 through the system into detector waste at a low rate 2.0-3.0 mL/min.

H. Toward the end of the water rinse, turn the injection valve at least 3 times into inject position for 30 seconds each time.

I. Prepare 1 L of 6.5 mM Na_2EDTA (must be the disodium form) and filter it through a 0.2 µm Nylon filter. Discard water from E1 and replace it by the filtered aliquot of 6.5 mM EDTA.

J. Pump at least 30 mL of EDTA into pump waste at priming flow rate.

K. Stop the pump flow. Close the priming valve. Pump (3 mL/min) at least 300 mL of EDTA from E1 through the system into detector waste.

L. Toward the end of the EDTA rinse, turn the injection valve at least 3 times into inject position for 30 seconds each time.

M. **Carry out steps (D)-(H) again**. Replace the water with 50 mM NaOH in E1. Prime the PTFE tubing with the new eluent aliquot.

N. Rinse the system flow path (column remains out of the system) with the initial eluent composition.

O. Stop the pump flow.

P. Open the working electrode side of the cell, remove the gasket and rinse the sealing surface with 18 MegOhm-cm water.

Q. Reassemble cell with a new disposable electrode or freshly polished conventional electrode and a gasket.

2. Parts and Start-Up for ED (ICS-3/5000) Electrochemical Cells

NOTE

This information is also available on the Disposable Electrode Installation Guide (Doc. No.: 065040) shipped with each order. Refer to Appendix A for ED40, ED50, or ED50A cells.

Table 3 Common Electrodes, part numbers and compatible gaskets

Electrode Type	Electrode Material	Electrode P/N	Compatible Gaskets	Gasket P/N
	Electrode Diameter: 1 mm			
Conventional	ICS-3/5000 Gold (Au)	061749	1 mil* Ultem® **	045972
	ICS-3/5000 AAA Gold (Au)	063722		057364
	ICS-3/5000 Silver (Ag)	061755	15 mil UHMWPE***	
	ICS-3/5000 Platinum (Pt)	061751		
	ICS-3/5000 Glassy Carbon (GC)	079854		
	ED50 Gold (Au)	044112		
	ED50 AAA Gold (Au)	055832		
	ED50 Silver (Ag)	044114		
	ED50 Platinum (Pt)	044113		
	ED50 Glassy Carbon (GC)	044115		
Disposable	Carbohydrates Gold (Au) [6/pk]	060139	2 mil Teflon® (Analytical)	060141
	Carbohydrates Gold (Au) [24/pk]	060216		
	AAA-Direct Gold (Au) [6/pk]	060082	1 mil* Teflon® (Capillary)	072117
	AAA-Direct Gold (Au) [24/pk]	060140		
	Silver (Ag) [6/pk]	063003		
	Platinum (Pt) [6/pk]	064440		
	Gold (Au) PTFE	066480	1 mil* Ultem®**	069339
	Carbon (C) PEEK	069336		
	Electrode Diameter: 3 mm			
Conventional	ICS-3/5000 Gold (Au)	063723	3 mil Teflon®	063537
			5 mil Teflon®	063550

* mil: One mil equals 1/1000 of an inch or 25.4 µm.
** Ultem: Polyetherimide (PEI).
***Ultra High Molecular Weight Polyethylene. This gasket was developed for use with gold electrodes

Table 4 Replacement Parts

P/N	Product Description
072042	ED Detector (No cell)
072044	ED Cell (no reference or working electrode)
062158	ED Cell Polypropylene support block for use with disposable electrodes
061879	pH-Ag/AgCl Reference Electrode
072162	Gasket for pH-Ag/Ag/Cl Reference Electrode (Capillary flow rates)
072075	Palladium Hydrogen Reference Electrode (Capillary IC only)
072214	Palladium Hydrogen Reference Electrode Gasket for ED cell (Capillary)
074221	7" Precision cut PEEK tubing kit - replacement for Ti tubing, post-ED cell (Capillary only)
060141	PTFE Gasket for Disposable Electrode, Pack of 4, ED/ED40/ED50/ED50A Amperometry Cell, 0.002 mil
045972	Ultem Gasket ED/ED50A/ED50/ED40 Amperometry Cell, 0.001 mil
063550	PTFE gasket for use with 3 mm gold electrodes, 0.005 mil
063537	PTFE gasket for use with 3 mm gold electrodes, 0.003 mil
069339	Ultem gasket for Carbon Disposable electrode ED/ED50A/ED50/ED40 Amperometry Cell, 0.001 mil
060356	ED50A Electrochemical Cell for LC25 and Spacer Block P/N 060297
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-Ag/AgCl Reference Electrode (ED50A)
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
057364	15 mil HDPE gasket for extending calibration range to higher concentrations for gold electrodes

* mil: One mil equals 1/1000 of an inch or 25.4 µm.
** Ultem: Polyetherimide (PEI).
***Ultra High Molecular Weight Polyethylene. This gasket was developed for use with gold electrodes

2.1 Reference Electrode Calibration Procedure

The following section is a detailed procedure for calibrating the pH/Ag/AgCl Reference Electrode used in the electrochemical cell of the ICS-3/5000. Calibration is necessary for obtaining a correct reference potential with "pH referenced" waveforms. The pH referenced waveforms are utilizing the potential of the pH/Ag/AgCl combination electrode . This procedure is not required for Ag/AgCl referenced waveforms and with the palladium hydrogen reference electrode. See Section 2.5 for initial use of the palladium hydrogen reference electrode. The pH calibration can be useful even with Ag/AgCl referencing if performed only once during initial installation of the reference electrode. Any subsequent changes of pH read out with an eluent composition of known pH value can be used for following a gradual change of the reference potential of Ag/AgCl electrode. The reference potential of Ag/AgCl slowly increases when the electrode is exposed to alkaline eluents.

1. Install the ICS-3/5000 Electrochemical Detector module into the DC without the ED flowcell.
2. Remove the pH Reference Electrode from the storage cap containing the saturated KCl solution, rinse the sensing end with DI water and gently dry it with a laboratory tissue. If the RE is being used at capillary flow rates on an ICS-5000, make sure to install the gasket into titanium block (P/N 072162) according to the ICS-5000 Installation Manual.
3. Plug the pH Reference Electrode into the ICS-3/5000 ED.
4. Power on the DC, verify USB communication and open the default Chromeleon panel tabset. Select the DC tab.
5. Click on the 'Calibration' button in the DC panel tab (Figure 10) to open the ED Wellness panel
6. Prepare a small container with pH 7.0 buffer. Support the pH Reference Electrode so that the pH Reference Electrode is immersed without holding or supporting it by hand (i.e. using a lab jackstand or boxes); ensure that the pH Reference Electrode body is immersed about halfway but avoid contacting the buffer with the pH Reference Electrode cable; if necessary, gently shake the electrode when immersed in the buffer to remove any trapped air bubbles on the surface of the electrode.
7. **After leaving the electrode a minute or two immersed in the pH buffer, observe the pH display in the Chromeleon Panel. The pH should remain stable for 5 seconds or longer (i.e., not vary by +/- 0.01 pH units), only then proceed with the calibration in step 8.** *It is critical for successful pH reference electrode calibration that the pH display is stable*. Do not stir or attempt to calibrate during this time.
8. Click on the 'Offset Cal.' button (Figure 11) to begin the slope offset (pH 7 buffer) calibration test; a message in the calibration screen and audit trail will indicate that the calibration has passed or failed.
9. If the calibration passed then proceed to the next step; if the calibration failed, make at least one more calibration attempt. If multiple attempts fail replace the pH Reference Electrode and repeat from Step 1.
10. Rinse the pH Reference Electrode in DI water and gently dry it with a laboratory tissue.

11. Prepare a small container with pH 10.0 buffer. Support the pH Reference Electrode so that the pH Reference Electrode is immersed without holding or supporting it by hand (i.e. using a lab jackstand or boxes); ensure that the pH Reference Electrode body is immersed about halfway but avoid contacting the buffer with the pH Reference Electrode cable; if necessary, gently shake the electrode when immersed in the buffer to remove any trapped air bubbles on the surface of the electrode.
12. **After leaving the electrode a minute or two immersed in the pH buffer, observe the pH display in the CM Panel. The pH should remain stable for 5 seconds or longer (i.e., not vary by +/- 0.01 pH units), only then proceed with the calibration in step 13.** *It is critical for successful pH reference electrode calibration that the pH display is stable*. Do not stir or attempt to calibrate during this time.
13. The pH slope (pH 10 buffer) calibration is performed by clicking on the 'Slope Cal.' button;
    a message in the calibration screen and audit trail will indicate that the calibration has passed or not passed.
14. If the calibration failed, make at least one more attempt. If multiple attempts fail replace the pH Reference Electrode.
15. If the calibration passed then proceed to step 16.
16. Install the pH Reference Electrode in the ED flowcell and connect the pH Reference Electrode to the ED.

Figure 10 Default panel tabset or ePanel for the EC Detector indicating the *Calibration* button

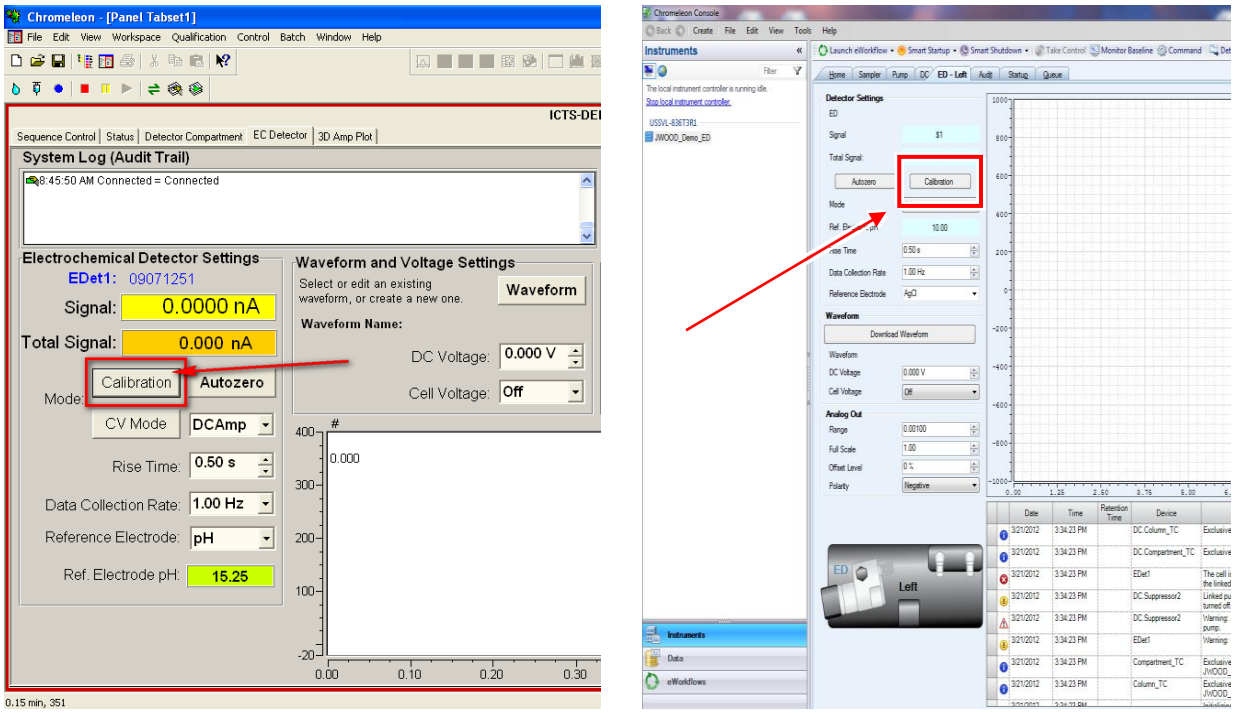
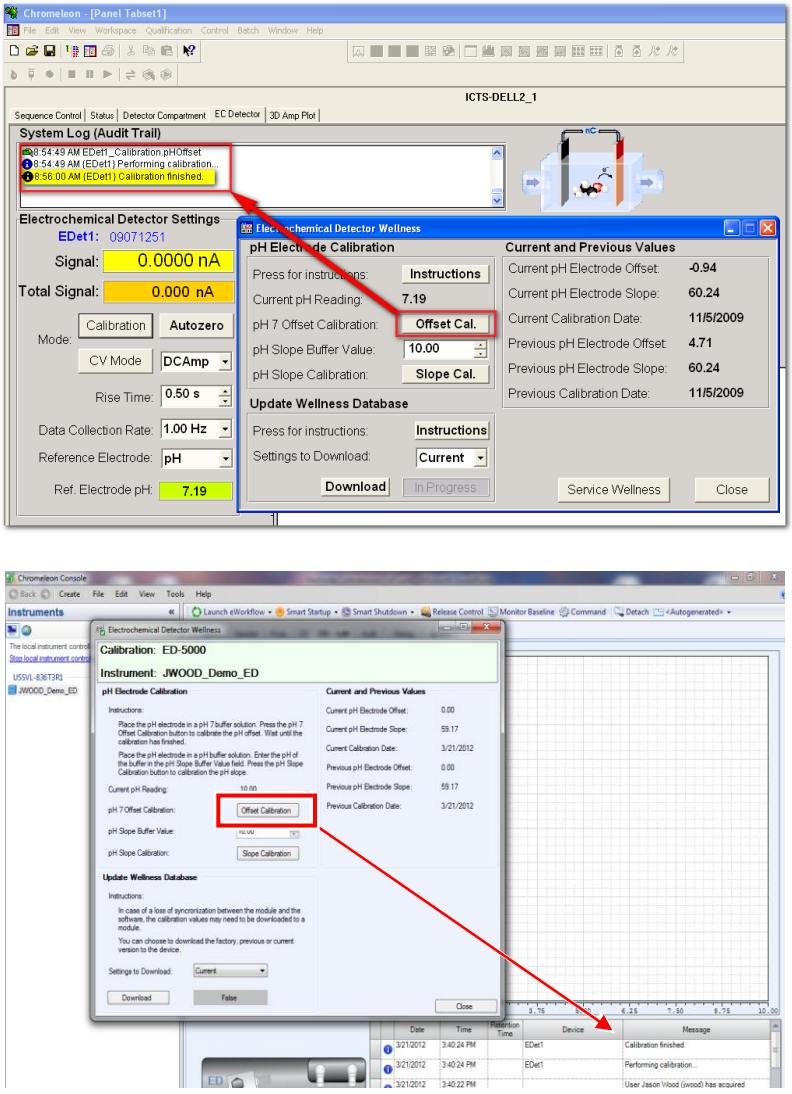


Figure 11 Example showing successful pH Offset Calibration (pH buffer 7) result



2.2 Palladium Hydrogen Reference Electrode Conditioning Procedure

This procedure is required for initial use of the PdH Reference Electrode to establish the hydrogen presence at the surface of the reference electrode. This procedure should be repeated if the PdH Reference Electrode is stored dry for an extended time (greater than 1 week). PdH Reference Electrode are only supported in Capillary IC (ICS-4/5000), this procedure is therefore written for a capillary system at capillary flow rates.

2.2.1.1 Install the PdH Reference Electrode according to the ICS-5000 Installation Manual, Section B.1.5

2.2.1.2 On the Chromeleon 7 ePanel Set or Chromeleon 6.8 panel tabset, select the following settings:

2.2.1.2.1 **Pump** panel: Set the pump flow rate to 0.01 mL/min. This turns on the pump flow.

2.2.1.2.2 **EG** panel: Enter 100 mM in the **Target Concentration** field. This turns on the power to the EGC. Turn on the CR-ATC.

2.2.1.2.3 Verify that eluent is exiting the ED cell.

2.2.1.2.4 **ED** panel: Set the reference electrode mode to **PdH**. Note: Older versions of Chromeleon may require a separate command for powering on the PdH electrode. Please check: Control/commands/ EDet/PdH Electrode Power

2.2.1.3 Condition the electrode for one (1) hour at these settings.

3. Electrodes and Applications

A broad range of electrochemically active molecules can be detected with good to excellent sensitivity using one of several forms of electrochemical detection. These molecules include: carboxylic acids; alcohols, glycols, aldehydes and carbohydrates; primary, secondary and tertiary amines; sulfoxides, sulfides, and mercaptans; and inorganic anions and cations. When compared to UV absorbance detection, electrochemical detection offers substantial improvements in sensitivity and selectivity for amine and hydroxy aromatics (catecholamines). Electrochemical detection also offers the advantage that carbohydrates can be detected without the derivatization required for fluorescence detection.

Thermo Fisher Scientific offers several choices of electrode products, including gold, platinum, silver and glassy carbon, which are all available in conventional or disposable formats.

Thermo Fisher Scientific also offers two types of disposable gold electrodes which differ only in the polymeric substrates for the working electrode material. The two substrate types are polyester (PE) and polytetrafluoroethylene (PTFE).

Conventional working electrodes (CWE) consist of a rod of the electrode material (gold, silver, platinum or glassy carbon) embedded in a polymeric support. When maintained properly, CWEs will last for extended periods of time, yielding consistent results and allowing the user the option to polish the electrode surface should the performance degrade over time or when performance diminishes as a result of multiple analyses. Thermo Fisher Scientific offers 1 mm and 3 mm conventional working electrodes depending on the application these electrodes are applied too. In general, as the area of the electrode goes up the signal increases, however noise also increases. Therefore, even though the 3 mm electrode will have a higher signal, it will also have a higher noise. The advantage of the 1 mm working electrode is that even though the signal has decreased because of the smaller surface area of the electrode, the random noise (electronic, temperature dependent…etc) decreases at an even faster rate. So, even though signal is slightly decreased, signal-to-noise is significantly improved in the 1 mm design.

Disposable electrodes, pioneered by Dionex (now a part of Thermo Fisher Scientific), are manufactured by physical vapor deposition of a thin layer of the working electrode material onto a thin polymer film. Care should always be taken when handling these electrodes to ensure that the working electrode is not scratched. Because the working electrode is a very thin deposition, the lifetime of the electrode is compromised by applying excessive voltages. Thermo Fisher Scientific offers specific waveforms or detection potentials developed for different applications and only specifies the lifetime of the disposable electrode when used under those conditions. Please see the Disposable Electrode Manual for waveforms recommended for each electrode material.

NOTE

Waveforms recommended for disposable electrodes may be used for CWE as well.

Table 5 Application Information

Disposable Electrode P/N	Conventional Electrode P/N	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: 044112 AAA: 055832 Gold ED (ICS-3/5000) Carbohydrates: 061749 AAA: 063722	Carbohydrates AAA Direct Carbohydrates AAA-Direct	CarboPac PA20 AminoPac PA10	Up to approx. 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 on PTFE: 066480		Carbohydrates and sugar alcohols#	CarboPac MA1	Up to 750 mM	N/A	4 week life time; no NaOH limit (tested to 750 mM).
N/A	3 mm Gold ED (ICS-3/5000) 063723	Aminoglycosides	Polymeric RP, Silica RP	0.5 M NaOH added post column	N/A	Post-column addition of hydroxide
Silver: 063003	Silver ED40/50 044114 Silver ED (ICS-3/5000) 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-3/5000) 061751	Alcohols and Chelating agents	IonPac ICE-AS1	N/A	100 mM	For higher MSA concentrations (above 100 mM) use conventional Platinum electrodes.
Carbon on PEEK: 069336	N/A	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.

Table 6 Typical Oxidation Applications with Various Types of Working Electrodes

Type of Working Electrode	Applications	Eluent Conditions
Gold	Carbohydrates, Amino Acids, Primary, Secondary, and Tertiary Amines, Aldehydes, Peroxides, Hydrazines, Nitrite, DNA Adducts, Thiols, Uric Acids and Similar Compounds	Alkaline
Gold	Thiols (Mercaptans), Sulfides, Disulfides and S-Containing Species	Acidic
Silver	Cyanide, Sulfides, Bromide, Iodide, Thiocyanate, Thiosulfate	Alkaline
Platinum	Alcohols, Glycols, Aldehydes, Sulfoxides, Thiols, Sulfides, Cyanide, Iodide, Ketones, Nitriles, Carboxylic Acids, Arsenite, Hypochlorite, Sulfite, Alkenes, Alkynes, Hydrazines, Carboxylic Chelating Compounds	Acidic
(Glassy) Carbon	Catecholamines, Aromatic Amines, Vitamines, Antioxidants, Nitrite, Sulfite, Iodide, Pesticides, Polynuclear Aromatics, Chlorinated Anilines, Flavonoids, Phenols and Phenolics	Acidic

4. Troubleshooting

The followings are examples of troubleshooting instruction for gold electrodes. For general troubleshooting tips for ED50A and ED electrochemical detectors please review the appropriate sections of the ED50A (Doc. No.: 031722) and ED manuals (Doc No.: 065342). For specific application troubleshooting, please review an appropriate column product manual which also provides detailed troubleshooting advice.

NOTE

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

4.1 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.

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

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 a new DE can be installed.

NOTE

Old style (three-pointed) pogos were used in cells prior to 2003.
The pogo problem described in Section 4.2 cannot occur with ICS-3/5000 cells.

4.3 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 Thermo Fisher Scientific 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.

4.4 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.

4.5 Low Signal Response Due to System Contamination

Low signal response not attributable to the electrochemical detector components may be due to system contamination.

Table 6 Possible Sources of System Contamination Leading to Low Signal Response

Source	Comments	Recommended Action
DI water	Poor quality DI water used for eluent preparation is often the cause of poor response. Minimum DI water purity requirement is ASTM Type I or better, 18.0 MegOhm-cm resistivity. The DI water should also be filtered through a 0.2 μm porous disposable nylon filter and be free ionic impurities, organics and particulate matter. It is not recommended to use HPLC grade or bottled water as these often do not meet the required specifications and can have a high CO_2 content.	Verify that the quality of the DI water meets the minimum purity requirements
Laboratory ware	In the preparation of eluents, cross-contamination from shared vessels may occur. Insufficiently cleaned labware will also be a contamination source.	Rinse the labware with 2 M NaOH followed by a DI water rinse
PEEK Tubing	The PEEK tubing used in the eluent pathway even if new may be contaminated	Rinse the PEEK tubing with 2 M NaOH followed by a DI water rinse
Reagents	Reagents that do not meet the purity requirements for electrochemical detector applications will result in poor response	Use only approved reagents meeting specified purity requirements
	NaOH: Never prepare sodium hydroxide solutions from pellets. The ideal preparation is from fresh 50% w/w NaOH solution. Ensure that the hydroxide reagent and solutions are minimally exposed to atmospheric CO_2 by handling under a blanket of inert gas such as N_2.	Order and use NaOH, Certified Grade, 55-254-500 or 033465
	NaOAc: use only Thermo Fisher Scientific-recommended sodium acetate meeting electrochemical grade specifications	
General Handling	Handling of detector components without gloves may result in contamination	Always use non-powdered gloves
Metal Ions	Any stainless steel surface in the wettable flow pathway may leach metal ions and result in metal contamination. This type of contamination will decrease signal response by complexing to the analyte or chelating to substrates.	Replace contaminated components and consumables. System cleansing may require a chelating agent such as oxalate or EDTA.
Microbial Growth	The presence of microbial agents may affect signal response. Ensure that the eluents are freshly prepared and microbial growth is not allowed to occur. This includes autosampler wash solutions, peristaltic pump reservoir, *etc.* are freshly prepared.	Rinsing the system with 2 M NaOH will ensure that all microbial contamination is removed