PQ

for DECADE Elite, Lite, I, II and Intro

171.0023P, Edition 9, 2017

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Introduction

This document describes the Qualification procedure as advised by the manufacturer. It is a result from our interpretation of many regulations and laboratory practices. In addition, feedback from users and representatives helped us to finalize this procedure.

In this document, all qualification checks have to be approved, or should be marked "n.a." if not applicable. Any deviation observed must be documented in the 'non-conformance' record. All relevant documents regarding this operational qualification must be filed together in one location.

As regulations and customer requirements may change, manufacturer reserves the right to introduce changes without prior notice. For details on functionality, operation and theory refer to the instrument user manuals.

Supported configurations

The PQ procedure in this document is applicable to an HPLC-ECD system with an Antec electrochemical detector (DECADE Elite™, DECADE Lite, DECADE II™, DECADE™ or INTRO™) and Antec flow cells with **glassy carbon** (GC) or **gold** (Au) working electrodes. Flow cells with working electrodes other than Au or GC are not supported with PQ procedures.

C H A P T E R   2

Identification

Engineer

The undersigned engineer certifies to be trained and qualified to perform PQ on Antec instruments.

Name .………………………………………………

Initials ……

Company .………………………………………………

……………… ……………..……………
Date Signature

Reviewer/customer

The undersigned reviewer/customer accepts that the above-mentioned engineer is trained and qualified to perform a PQ on Antec instruments.

Reviewer/Customer .…………………………………………………

Initials ……

Job title .…………………………………………………

Company & Dept. .…………………………………………………

……………… ……………..……………
Date Signature

Instrument description

Antec detector and flow cell(s)

☐   DECADE Elite, Lite,   p/n: ……………   s/n: ……………
I, II or Intro

☐   Detector has DCC option (Y/N) ………….

☐   Flow cell*   p/n: ……………   s/n: ……………

Working electrode type: ……………

Reference electrode type: ……………

*s/n: entering more than one s/n is allowed for DCC detectors.

(U)HPLC instrument

☐   Pump*   ……………   s/n: ……………

☐   Autosampler/injector   ……………   s/n: ……………

☐   Degasser   ……………   s/n: ……………

☐   Acquisition software &   …………………………………………….
revision nr

*s/n: entering more than one s/n is allowed for set-up with DCC detectors.

Verified by (customer): …………………………..   Deviations (Y/N): ……

Comments:

Test materials

☐   Standard*   …………………………..

Lot nr   …………….   Exp. date   …………….

☐   Mobile phase   …………….   …………….

* Fill in 'glucose' or 'MOPEG'

Test devices

☐   Dummy cell*   p/n: 250.0040   s/n: …………….

☐   Volt meter or AD signal   p/n: …………….   s/n: …………….

*s/n: entering more than one s/n is allowed for DCC detectors.

Other relevant information

Description	

Verified by (customer): …………………………..   Deviations (Y/N): ……

Comments:

C H A P T E R   3

PQ test

Principle

For testing the performance of the HPLC/ECD sys-
tem, a test substance is injected onto a Flow Injec-
tion Analysis (FIA) tubing assembly (, and repeata-
bility, linearity & peak height is measured.

Different detection modes for GC and Au work-
ing electrodes

In case of the use of a flow cell with glassy carbon
electrode, the detection method is set to direct cur-
rent (DC) mode, which will apply one working poten-
tial. In case of testing with a gold working electrode,
Pulsed Amperometric Detection (PAD) mode is
used.

*Fig. 1. PQ tubing kit installed in detector
oven and connected to flow cell.*

Test parameters

Important parameters to characterize and check the performance of all relevant
parts of a detection system are:

- Repeatability
- Linearity
- Detector response
- Background current (only with GC electrode)
- Signal-to-noise ratio (only with GC electrode)

Not only the detector and flow cell performance but also those of pump and au-
tosampler are taken into account with these parameters. In case of testing the per-
formance with a gold flow cell, the background current, noise and S/N measure-
ment are not relevant, as in PAD mode the signal is digitally processed.

As the detector response is affected by electrode size and injection volume, differ-
ent values are specified for different combinations of hardware.

For Clarity software, pre-configured PQ method files are available on our website
for download.

Parts and consumables

To perform the PQ, a (reusable) PQ FIA tubing kit and PQ consumables kit are necessary (Table 1). Two different PQ consumables kits are available: one specific for testing glassy carbon electrodes (pn. 250.3048), and another specific for gold working electrodes (pn. 250.3046).

Table 1. Contents of Antec PQ kits

Part	Part no	Qty
PQ FIA tubing set	**250.1052**	
Tubing assembly for Flow Injection Analysis		1
PQ consumables, ECD in DC mode	**250.3048**	
Concentrated buffer for PQ	250.1064	1
MOPEG 4.0 µmol/L, 2 mL for PQ	250.1062	4
Document: PQ for D2 Elite Lite and ROXY	171.0023P	1
Document: OQ for D2 Elite Lite and ROXY	171.0023O	1
PQ consumables, ECD on Au at pH 13	**250.3046**	
Glucose for PQ (dry powder; for 3 mL stock solution of 20 mM)	250.1067	1
Document: OQ for D2 Elite Lite and ROXY	171.0023O	1
Document: PQ for D2 Elite Lite and ROXY	171.0023P	1

Additional consumables and chemicals that are necessary to perform the PQ procedure are:
- Autosampler vials
- 1 L ultra-pure water (resistivity > 18 MOhm.cm, low TOC)
- 50 mL methanol, HPLC-grade or better (only for test in DC mode on GC)
- Sodium hydroxide 50% solution, HPLC-grade (only for test in PAD mode on Au)

Preparations

For a successful performance test it is important that the HPLC-EC system has been optimized and is in top condition.

OQ

Make sure to have performed the Operation Qualification (pn. 171.0023O) before performing the PQ test. Results of the OQ are to be summarized in the PQ results table.

Flush the system

1. Prepare a bottle with degassed water and use it to flush the system up to the injector.
2. Connect the red-striped end of the PQ FIA tubing directly to the valve. If needed use the 1/32"OD tubing adapter.
3. Flush the FIA tubing for 10 min with water at 1 mL/min.
4. Prepare the mobile phase according to Table 2.
5. Connect the mobile phase and run about 30 mL through the system.

Install the flow cell and let the signal stabilize

6. Clean the flow cell (see user manual) and assemble with a 50 µm spacer.
7. Connect the flow cell to the orange striped end of the FIA tubing set using the correct connectors, and ensure that the flow cell is filled air-free with mobile phase.
8. Set the detector conditions as given in Table 2 and turn on the cell.

⚠️ WARNING

Do not turn the cell on when mobile phase has not yet completely filled the cell. Turning on a cell that still has air inside can damage the cell!

9. Wait for the system to stabilize for at least 30 min before starting the PQ HPLC tests.

Prepare the sample

10. For testing flow cells with glassy carbon electrode only: the MOPEG solution is a ready to use standard and only needs to be diluted in case the linearity check is done with diluted standard instead of injection volume variation.
11. For testing flow cells with gold electrode only: Glucose needs to be dissolved and diluted:
    - Add 3 mL of mobile phase to the bottle with Glucose and shake to dissolve thus making a 20 mM stock solution.
    - Dilute the 20 mM stock solution 1000x with MP. For example, dilute in 3 consecutive steps: mark 3 empty vials clearly as "1", "2", "3" and add 900 µL mobile phase to each of the 3 vials. Add 100 µL stock solution in vial 1, close and shake well. With a new clean pipet tip take 100 µL solution from vial 1 and add to vial 2, close and shake. With a new clean pipet tip take 100 µL solution from vial 2 and add to vial 3. Vial 3 now contains 1 mL of 20 µmol/L Glucose standard ready for a PQ test injection.

For gold cells the PQ standard solution is 20 µmol/L Glucose in mobile phase (MP). Dissolve and dilute the glucose powder prior to use.

HPLC-ECD test conditions for PQ

Table 2. HPLC-EC conditions for PQ.

General settings	
PQ hardware	PQ FIA tubing set (250.1052)
Flow rate (signal)	1 mL/min (up to 1.5 mL/min is allowed)
Temperature	35 ˚C (flow cell and FIA tubing set inside the detector oven)
Integrator	Data acquisition frequency: < 5 Hz
Flow cell spacing	50 um spacer ,or AST setting '2' for SenCells
Settings for testing with <u>glassy carbon</u> working electrodes	
Mobile phase	1 bottle concentrated PQ buffer (pn. 250.1064), 50 mL MeOH (HPLC grade or better) and water (18 MOhm.cm, low TOC) mixed to a final total volume of 1000 mL. PQ buffer already contains chloride required in case of using an ISAAC.
Sample	MOPEG 4 µmol/L (pn. 250.1062)
E cell	+610 mV (vs HyREF); +800 mV (vs sb); +650 mV (vs ISAAC)
Filter	0.1 Hz
Range (for signal)	200 nA/V for 2 mm, 3 mm and Flexcell GC electrodes 10 nA/V for 0.7 mm GC electrodes
Range (for noise)	1 nA/V
Flow rate (for noise)	0 mL/min or set to an optimum flow rate
Settings for testing with <u>gold</u> working electrodes	
Mobile phase	100 mM NaOH in water (HPLC grade, low TOC, and >18 MOhm.cm)
Sample	20 µmol/L Glucose in mobile phase
E (PAD mode)	<u>For DECADE Elite:</u> E1, E2, E3, E4 = 100, -2000, 600, -100 mV (E5 0 mV); t1, t2, t3, t4, ts = 400, 20, 10, 70, 200 ms (t5: 0 ms) <u>For DECADE II:</u> E1, E2, E3 = 100, -2000, -100 mV t1, t2, t3, ts = 400, 20, 80, 200 ms Icell is about 0.5 - 2 µA
Filter	off
Range	1 µA/V

Specific settings for DECADE I and INTRO (GC electrodes only)	
Noise filter	Signal: 0.1 s; Noise: 1 s
Noise analysis	DECADE: pA range; INTRO: att x100 = off

PQ procedure

Test injection

Run a test chromatogram with the test solution. Check the test-chromatogram peak time, and optimize the automated integration parameters if necessary.

In case of using a 20 or 100 µL sample loop on the injector: adjust the detector range if the test peak height is larger than 50% of the full scale range. The analyses for linearity require a larger injection volume and the signal may run off scale if the range setting is too low.

Linearity test

A 5-point calibration curve has to be set up for linearity evaluation. Data can be generated by varying the concentration or injection volume:

- Varying concentrations: dilute the test solution by hand thus generating equidistant <u>concentrations</u>, for example: 0.8, 1.6, 2.4, 3.2 and 4.0 µmole/L MOPEG (for gold cells: 4, 8, 12, 16, 20 µmole/L Glucose).

- Varying injection volumes: inject 5 different <u>volumes</u> of the test solution. For example: 4, 5, 6, 7, 8 µL (20 µL loop) or 20, 25, 30, 35, and 40 µL (100 µL loop).

The latter is the most preferred method because it eliminates manual dilution errors.

Repeatability test

Repeatability is evaluated based on 8 subsequent analysis of the test solution.

Noise measurement and background current (for GC electrodes only)

Check the cell current (**I cell**) at the detector (e.g. read from the display) and write it in the Results Summary table (page 10).

Record a 15 min baseline trace using the setting specified in Table 2. When pump is suspected to contribute to the noise level, the pump may be switched off or set to another optimum flow rate.

Processing the PQ results

The PQ procedure results in linearity data, 8 repeatability chromatograms, and a baseline trace (for GC electrodes only).

1. Select the 8 'repeatability' chromatograms and create a report containing retention time, peak heights and peak areas. Calculate the average values and the relative standard deviation as percentage of the average (%RSD).
2. Process the linearity data and calculate the correlation coefficient between peak area and concentration or injection volume.

Write the results down in the PQ results table on page 11

Contact your supplier if the final result of the HPLC PQ procedure is 'failed'. Note also that a 'failed' PQ is not necessarily related to the detector.

Additional parameters for GC electrodes:

3. Open the baseline trace and evaluate the noise as described in the OQ document (171.0023O), or use any other software that is capable of the calculating the baseline peak-to-peak noise level (ASTM).
4. Calculate the S/N ratio (=peak height/noise level) after obtaining the noise value. The average peak height from the repro-evaluation is used as "signal".

Write the results down in the PQ results table on page 11.

> **To expand the life time of a PQ tubing set flush it with water before storage.**
>
> **Before flushing the system, SWITCH OFF AND REMOVE THE CELL!**

Recalculation to the correct unit - signal in mV or nA

In case the detector is connected to an AD convertor, this will usually generate a signal with the units in mV. Use the detector range setting to convert to current.

Range (nA/V) * Signal (mV) = Signal (pA)

For example, at the 2 nA/V range, a signal of 750 mV is actually a current of:

2 nA/V * 750 mV = 1500 pA (1.5 nA)

> **Use the proper units nA or pA by multiplying mV with range if necessary!**

C H A P T E R   4

OQ-PQ results summary

Electronic test results and HPLC test results

	Specified[*1]		Measured		Result[*2]
ELECTRONIC TESTS[*3]					
Dummy cell test					
Current (I-cell)	2.67 ± 0.05	nA	…….	nA	………….
Noise p-p	< 2.0	pA	…….	pA	………….
Analog output test					
Output at 5 nA/V	530 ± 10	mV	…….	mV	………….
HPLC TESTS					
Chromatogram					
Peak time	< 3	min	…….	min	………….
Response					
Height	> ………....	nA	…….	nA	………….
Repeatability					
%RSD t	< 0.5	%	…….	%	………….
%RSD area	< 3.0	%	…….	%	………….
Linearity					
Correlation coefficient r	> 0.997		…….		………….
Tests for GC WE only[*4]					
I-cell	< ....…	nA	…….	nA	………….
Noise	< ....…	pA	…….	pA	………….
S/N ratio	> ……….		…….		………….

*1 Specifications for some of the HPLC tests are hardware dependent: check the applicable specs on
    page 13 and copy the specified value into this table.

*2 Fill in 'passed' or 'failed'

*3 for 'electronic test' results copy the measurement values from the OQ test.

*4 Tests for background current and sensitivity are applicable only in case of using a glassy carbon
    working electrode in the flow cell. When testing with a **gold** working electrode, fill in n.a.

Final result[*2] _____

Verified by (customer): …………………………..   Deviations (Y/N): ……

Comments:

C H A P T E R   5

OQ-PQ results summary of optional parallel 2nd cell

For 2-channel configurations only, otherwise fill in n.a. (not applicable)

	Specified[*1]		Measured		Result[*2]
ELECTRONIC TESTS[*3]					
Dummy cell test					
Current (I-cell)	2.67 ± 0.05	nA	…….	nA	………….
Noise p-p	< 2.0	pA	…….	pA	………….
Analog output test					
Output at 5 nA/V	530 ± 10	mV	…….	mV	………….
HPLC TESTS					
Chromatogram					
Peak time	< 3	min	…….	min	………….
Response					
Height	> ………....	nA	…….	nA	………….
Repeatability					
%RSD t	< 0.5	%	…….	%	………….
%RSD area	< 3.0	%	…….	%	………….
Linearity					
Correlation coefficient r	> 0.997		…….		………….
Tests for GC WE only[*4]					
I-cell	< ….....….	nA	…….	nA	………….
Noise	< ………….	pA	…….	pA	………….
S/N ratio	> ……….		…….		………….

*1 Specifications for some of the HPLC tests are hardware dependent: check the applicable specs on page 13 and copy the specified value into this table.

*2 Fill in 'passed' or 'failed'

*3 for 'electronic test' results copy the measurement values from the OQ test.

*4 Tests for background current and sensitivity are applicable only in case of using a glassy carbon working electrode in the flow cell. When testing with a **gold** working electrode, fill in n.a.

Final result[*2] _____

Verified by (customer): …………………………..   Deviations (Y/N): ……

Comments:

C H A P T E R   6

Hardware dependent test specifications

HPLC-ECD with 20 - 100 µL sample loop

Test injection volume: 20 µL

Parameter	specified			
WE ID	**0.7 mm**	**2mm**	**3mm**	**Flexcell**
peak height, MOPEG Glucose on gold	> 0.8 nA > 5 nA	> 4 nA > 25 nA	> 8 nA > 50 nA	> 10 nA > 100 nA
I cell noise p-p S/N ratio	< 3 nA < 2 pA > 4000	< 8 nA < 6 pA > 4000	< 12 nA < 8 pA > 4000	< 20 nA < 14 pA > 2000

HPLC-ECD with 5 - 20 µL sample loop

Test injection volume: 5 µL

Parameter	specified			
WE ID	**0.7 mm**	**2mm**	**3mm**	**Flexcell**
peak height, MOPEG Glucose on gold	> 0.2 nA > 1.3 nA	> 1 nA > 6.3 nA	> 2 nA > 13 nA	> 2.5 nA > 25 nA
I cell noise p-p S/N ratio	< 3 nA < 2 pA > 1000	< 8 nA < 6 pA > 1000	< 12 nA < 8 pA > 1000	< 20 nA < 14 pA > 500

HPLC-ECD with 2 µL sample loop

Test injection volume: 2 µL

Parameter	specified			
WE ID	**0.7 mm**	**2mm**	**3mm**	**Flexcell**
peak height, MOPEG	> 0.1 nA	> 0.5 nA	> 1 nA	> 1 nA
I cell	< 2 pA	< 6 pA	< 8 pA	< 14 pA
noise p-p	< 2 pA	< 6 pA	< 8 pA	< 14 pA
S/N ratio	> 500	> 500	> 500	> 250

C H A P T E R   7

PQ certification

The Performance Qualification (PQ) tests were carried out in accordance with the PQ procedure and have been carried out to the satisfaction of both parties.
All tests as described in this document have been successfully completed, and all results are within specifications.

Antec representative

Company   …………………………………………………….

Performer   …………………………………………………….

………………   ……………..……………
Date   Signature

Customer (authorized to sign)

Company & Dept.   …………………………………………………….

Reviewer/Customer   …………………………………………………….

………………   ……………..……………
Date   Signature

Comments

Verified by (customer): …………….…………..   Deviations (Y/N): ……

Comments:

C H A P T E R 8

Non-conformance record

Any case of non-conformance found during the qualification procedure should be documented and signed for acceptance or corrective action taken.

Ref.	Non-conformance and action taken	Signature customer	Sign. executing technician
1		………………	………………
2		………………	………………
3		………………	………………
4		………………	………………
5		………………	………………
6		………………	………………