

Thermo QExactive Instrument Card

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Overview

QCloud is an automatic quality control tool with minimal user intervention that has been designed to assess the performance of the LC-MS/MS systems in proteomics laboratories.

Samples used for quality control are injected using a defined naming system, and a program named QCrawler automatically sends the files to the QCloud FTP server. Extracted information is available in the QCloud web server (<http://qcloud.crg.eu>) in which parameters such as global numbers (#id proteins, #id peptides) as well as specific values for selected peptides (area, retention times, resolution) are represented in an interactive graphical display.

Samples used for quality control in the QCloud

Two types of quality control samples have been established according to the nomenclature and purpose introduced by Karl Mechtler (Pichler et al 2012).

QC1 Sample: Sample of low complexity. To establish the performance of the instrument before and after each sample or batch of samples (several times per day).

QC2 Sample: Sample of high complexity. To establish the performance of the instrument with a complex sample that mimics real samples. Injected at least once per week, ideally 3-5 times per week. QC2 sample is now a HeLa digest but we are currently evaluating the use of a human digest that contains also isotopologues from Promega (6 × 5 LC-MS/MS Peptide Reference Mix) to additionally evaluate the dynamic range of the instrument.

The QC samples are commercially available to minimize variability. QCloud users should use the following standards:

QC1 Sample

Product: Trypsin-digested BSA MS Standard (CAM modified)

Part number: P8108S

Price: 83€ (1 x 500 pmol)

Company: New England Biolabs

QC1 sample preparation: The commercial product is a vial of 500 pmol dried digested BSA. Dissolve the vial in 500 µL 0.1% FA. Then dilute the BSA to 50 fmol/µL (15 µL stock + 285 µL 0.1%FA) and inject 0.5 µL (25 fmol).

QC2 Sample

Product: Pierce HeLa protein digest standard

Part number: 88329

Price: 365€ (5 x 20 µg)

Company: Thermo Fisher Scientific

QC2 sample preparation: The commercial product is a vial of 20 µg dried digested HeLa extract. Dissolve the vial in 200 µL 0.1% FA (100 ng/µL). Inject 1 µL (100 ng).

QC Methods

QCloud system is method-independent but peak width and cycle times are important parameters in order to guarantee the good performance of the peak picking algorithms.

We suggest injecting QC1 samples in a short method in order to save instrument time. Specifically we recommend a gradient length of 8 min (total acquisition time 25 min). The chromatographic peak width in this method is narrow and standard MS methods need to be changed to TOP3 for Orbitrap QExactive instruments to keep the cycle time within the optimal range. The method used to analyze the QC2 samples can be any standard method used to analyze high-complexity samples acquired in shotgun data-dependent mode and our recommendation for gradient length is 60 min (total acquisition time 80 min). In both QC methods we suggest to deactivate the "lock mass option" to be able to detect deviations in the mass accuracy.

Find below the details of the MS methods and chromatographic methods that are given as suggestion but can be adapted depending on the chromatographic system and the column used.

QC1 Samples

MS Method:

- *Acquisition: DDA Top3*
- *Resolution in MS1: 70000*
- *Resolution in MS2: 35000*
- *Acquisition time: 25 min*
- *No lock mass*

LC Method:

- *Flow 300nL/min*
- *Gradient (A: 0.1%FA; B: 80%ACN 0.1%FA)*

<i>Time (min)</i>	<i>%B</i>
0	6
1	6
9	56
10	100
25	100

QC2 Samples

MS Method:

- *Acquisition: DDA Top10*
- *Resolution in MS1: 70000*
- *Resolution in MS2: 35000*
- *Acquisition time: 80 min*
- *No lock mass*

LC Method:

- *Flow 300nL/min*
- *Gradient (A: 0.1%FA; B: 80%ACN 0.1%FA)*

<i>Time (min)</i>	<i>%B</i>
0	5
52	25
60	40
70	100
80	100