POLYQUANT

EcoCAT

User Guidelines

Content

Kit content (for 50 applications):

Component	Amount (mol)
PQ-Eco-1	50 pmol
PQ-Eco-2	50 pmol

Reconstitution

The QconCATs are soluble in 50 mM ABC buffer and denaturing buffers containing urea or guanidinium-hydrochloride.
We recommend dissolving the content of the vial by adding 500 µl 50 mM ABC buffer pH 8.0, followed by 5 min incubation at RT and briefly mixing (e.g. Vortex) or pipetting up and down (10x).

Sample preparation

QconCATs are added directly to your analyte sample prior to sample preparation (reduction, alkylation, digestion). We recommend to add ≥ 10 nM of QconCAT to your analyte and proceed with your routine sample preparation procedure.

a) With *E. coli* background

- Spike 10 µg of *E. coli* Lysate with 1 pmol of the EcoCAT
- Digest with trypsin, using your standard protocol
- Inject a volume corresponding to 1 µg of *E. coli* lysate + 100 fmol of EcoCAT into your MS system

b) Without background

- After re-solubilisation, add ~1 ng of trypsin to EcoCAT
- Digest for 4 h or overnight at 37°C
- desalt the sample on a C18 tip/spin column
- Inject a volume corresponding to 100 fmol of EcoCAT into your MS system

MS measurement

The required amount of reference standard depends on instrumentation. For LC systems used in nanoFlow (< 1µl/min), we recommend using 500 fmol QconCAT on column per injection as a starting point.

Instrument settings

For initial testing, we recommend to run the samples in DDA mode. EcoCAT1 and EcoCAT2 include peptides ranging from 760 - 2222 Da. To be able to detect all peptides in DDA mode, your instruments need to include precursors between 380 m/z and 805 m/z.

Alternatively, you can run an unscheduled PRM or MRM. The target list is available on our website.

Example gradient (Buffer B = 80% ACN, 0.1% FA) used for a Vanquish Neo uHPLC, equipped with a 15 cm EasySpray column (ES900):

Time [min]	Buffer B [%]
0	1
3	6
38	20
56.9	35
57.4	55
58.1	99
59.6	99

Data analysis

For correct peptide identification, the peptide masses of each ^{14}N in every amino acid of all peptides is replaced by ^{15}N. This calculation is fully supported by software like Skyline or Spectronaut. You can find a preconfigured Skyline file with all peptides on our website. This file also contains a library that was generated on our instrument (Vanquish Neo coupled to an Orbitrap Exploris 240). You may want to delete this library and replace it by your own, as you may observe different fragmentation patterns and retention times.

Alternatively, you can find the exact masses of all precursors (heavy and light) here in the target list.

Feedback

If you need help with setting up the method, please do not hesitate to contact us at info@polyquant.com.

We would be happy to receive your feedback!