

## RePLiCal – a calibrant protein for retention time standardization in proteomics

RePLiCal facilitates comprehensive standardization of LC-MS<sup>1</sup> workflows in proteomics studies. As a calibrant protein operating at the peptide level, RePLiCal is specifically designed for retention time standardization and normalization in reverse-phase LC-MS analysis. Simple to be implemented into the user's workflow, RePLiCal directly works with your LC-MS setup and operates independently of additional software packages.

Enhanced normalization of reverse-phase chromatography separation between LC-MS runs

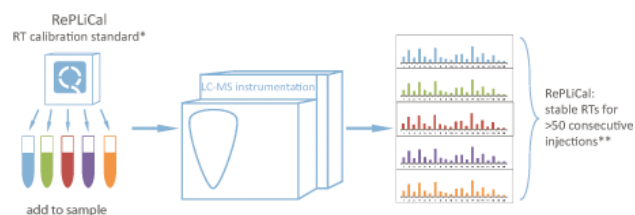
Accurate prediction of normalized retention time, even over extended analysis time

Assessment of the trapping column performance

Recalibration of the *m/z* scale post-acquisition

## Assess your LC performance

RePLiCal demonstrates high repeatability in RT measurements even over prolonged analysis time. With RePLiCal, deviations in RT and peak width from precise analyses allow for rapid feedback and real-time surveillance of your LC instrumentation.



Monitoring of LC performance longitudinally by RePLiCal

\* Predigested or intact RePLiCal protein

\*\* Average peak width <6%; RT RSDs<sup>3</sup> <1.2% for all RePLiCal peptides after 60 consecutive injections of 50 fmol RePLiCal in 1 µg of yeast tryptic peptides and analyzed using a 30 min LC gradient.

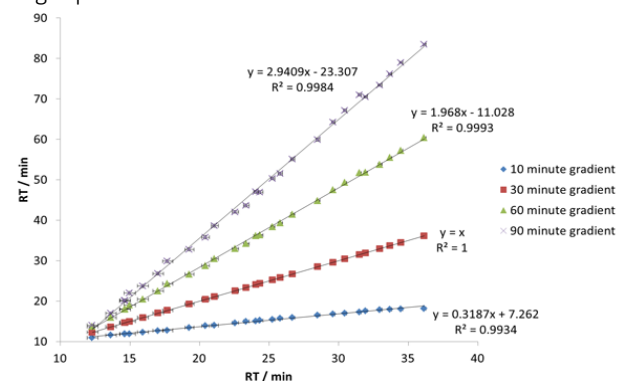
<sup>1</sup> LC-MS, liquid chromatography-mass spectrometry

<sup>2</sup> RT, retention time

<sup>3</sup> RSD, relative standard deviation

## Standardize LC-MS workflows

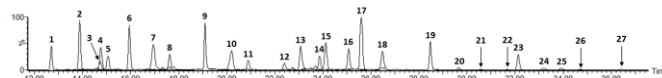
Having a high degree of linearity, RePLiCal enables the prediction of RTs of peptides on a new gradient. By calculating the regression fit, RTs of RePLiCal peptides characterized on the respective gradients can be extrapolated to predict RTs of target proteins.



RTs of RePLiCal peptides on different length LC gradients (3-40% 0.1% formic acid in acetonitrile) are compared using the 30 min gradient as a reference. Triplicate measurements; error bars: ±2 standard deviations. High confidence in linearity is also given for very long gradients (even for 480 min).

## Simplify your targeted analysis

Adding RePLiCal along with the sample saves overall operating time while enhancing data quality. When spiked into a tryptic digest of a whole cell yeast lysate and analyzed by nLC-nESI-SRM-MS, all 27 peptides are detected with good signal-to-background ratio even with only 5 fmol of RePLiCal.

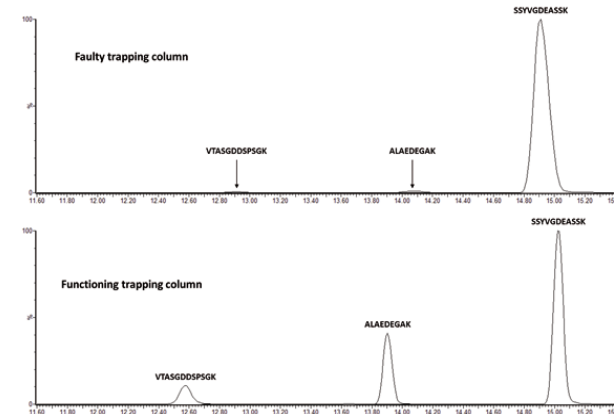


Chromatogram of a 30 min gradient (3-40% 0.1% formic acid in acetonitrile) of 5 fmol RePLiCal spiked into a whole cell yeast lysate tryptic digest (1 µg)

- readily detectable in a complex matrix
- only low amounts needed – does not increase the complexity of the sample
- high reproducibility
- benchmark RP LC instrument performance across laboratories
- optimization of LC gradient to maximize peptide and protein identification rates

## Test trapping column performance

RePLiCal contains two highly hydrophilic peptides, enabling the confirmation of full functionality of the trapping column.



Comparison of trapping column performance using the intensities of the three earliest eluting peptides in RePLiCal

## Ordering information

Download the order form at [www.polyquant.com/calibration-standards-for-proteomics](http://www.polyquant.com/calibration-standards-for-proteomics) and return the completed form by E-mail or fax.

Alternatively contact us by E-mail: [info@polyquant.com](mailto:info@polyquant.com) or call us at +49 9405 96999 10.

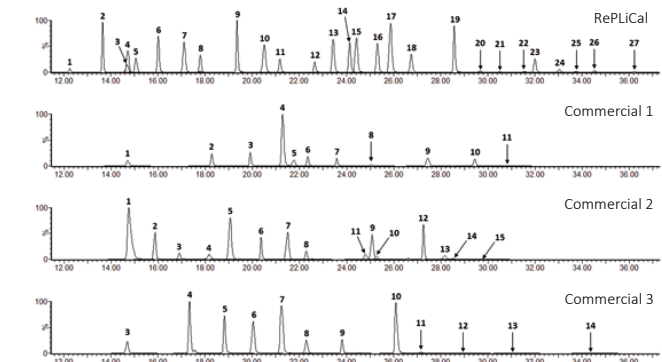
Type	Cat-No
RePLiCal complete protein 1 µg, lyophilized, undigested	PQ-CS-1560
RePLiCal peptide mix 1 µg, lyophilized, digested	PQ-CS-1561

For other package sizes please contact us.

## Extended coverage of RTs

RePLiCal's 27 lysine-terminating peptides were selected for good elution profiles, uniqueness across eukaryotic proteomes and range from highly hydrophilic to hydrophobic covering a broader range of retention times.

RePLiCal has a greater density of calibration points across the chromatographic gradient than other commercially available standards. Analyzing a whole cell yeast lysate digest (90 min LC gradient) only very few of the identified peptides (<2.4%) eluted outside of the retention times covered by RePLiCal.



Comparison of chromatograms on a 30 min LC gradient (3-40% 0.1% formic acid in acetonitrile) with nESI-SRM-MS data acquisition for RePLiCal and three commercially available retention time standards. The numerical annotations represent the elution order as provided by the manufacturer.

- more calibrant points than other commercial RT kits
- wider range of elution times
- realign data in label-free quantification studies
- real-time correction of time-scheduled SRM experiments

Reference: Holman S., McLean L. and Evers CE. RePLiCal – A QconCAT Protein for Retention Time Standardization in Proteomics Studies, J Proteom Res., 2016 Mar 4;15(3):1090-1102.