

QUICK REFERENCE CARD



Before starting with the protocol: Add 20 μ l of Acetonitrile (CAS no. 75-05-8) to the vial of Dissolution Buffer (blue cap) and mark the according checkbox.



1. Add 50 μ l Dissolution Buffer (blue cap) to the iRT Standard tube (yellow cap)
2. Vortex to mix, sonicate 5 minutes if possible
3. This solution is the 10x iRT Standard
4. Store 10x iRT Standard at 2 - 8 $^{\circ}$ C and use within 12 weeks



Add 10x Standard Peptide Mix 1:10 to your injection-ready peptide sample



Determine iRT values of target peptides:

1. Measure your sample in MRM/PRM or shotgun mode
 - a) for MRM/PRM add transitions/precursors for iRT peptides to your instrument method
 - b) for shotgun add FASTA file of iRT peptides to your search
2. Extract retention times (RT) for each iRT and target peptide with the software of your choice
3. Use the tools available at <https://biognosys.com/shop/iRT-Kit> to perform RT - iRT conversions and calibrate your LC system



Biognosys Software (e.g. Spectronaut, SpectroDive and Quic) will automatically perform RT - iRT conversions and utilize the data derived from the iRT kit in your sample for optimal performance

GENERAL INFORMATION

iRT - indexed Retention Time - is a dimensionless number characterizing chromatographic retention of a peptide in defined resin (e.g. C18) relative to the iRT Standard. In contrast to retention time the iRT value of a peptide is stable and therefore can be used to predict retention time on any chromatographic setup¹.

The iRT Kit contains Dissolution Buffer (blue cap) and iRT Standard (yellow cap) for use in time resolved mass spectrometry.

The iRT Standard contains eleven non-naturally occurring synthetic peptides in a pooled mix that serves to normalize retention time of other peptides to the iRT scale. The iRT peptides are distributed along a linear gradient. Please be aware that depending on your chromatographic settings not all iRT peptides might be detected. This does not impact the retention time normalization and calibration of your system.

The iRT peptides are the ideal reference set to quality control (QC) the LC-MS set-up over time. Relevant QC parameters can be automatically extracted from samples containing spiked-in iRT peptides with QuiC, freely available at: <https://biognosys.com/shop/QuiC>

Further information, such as the transitions for iRT peptides, the peptide sequences and the corresponding FASTA file can be downloaded at: <https://biognosys.com/shop/iRT-Kit>

¹ Escher C, Reiter L, MacLean B, Ossola R, Herzog F, Chilton J, MacCoss M.J, Rinner O: Using iRT, a normalized retention time for more targeted measurement of peptides, Proteomics 2012, 12(8): 1111-1121