Protocol

SpikeTides™ PTM-Kits and PTM-Peptides – quantified

Collection of 142 absolutely quantified proteotypic peptides for direct usage in mass-spectrometry based proteomics (MRM). The collection comprises wild-type peptides as well as 23 different posttranslational modifications (PTMs).
Each peptide is absolutely quantified and supplied in a separate vial.

Contact us:

InfoLine:	+49-30-6392-7878
Order per fax:	+49-30-6392-7888
or e-mail:	peptide@jpt.com
www:	www.jpt.com

JPT Peptide Technologies GmbH
Volmerstrasse 5 (UTZ)
12489 Berlin
GERMANY

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1 Introduction

One highly efficient method for the multiplexed detection and quantification of proteins like metabolic enzymes is targeted proteomics by mass spectrometry. The method makes use of one or more proteotypic peptide(s) from protein(s) of interest that are generated by tryptic digestion of the protein(s) and monitored by a selected reaction monitoring (SRM) or by a multiple reaction monitoring (MRM) assay.
Relative and absolute quantification by targeted proteomics requires stable isotope-labeled proteotypic peptides as internal standards. For absolute quantification these standards need to be absolutely quantified. The traditional way to prepare the quantified standards is the purification of the peptide to a very high level enabling subsequent amino acid analysis or alternative peptide quantification methods (LavaPep, Ninhydrin, Lowry) for peptide content determination. The drawbacks of these methods are low throughput and high costs for a) consumables for peptide synthesis (especially for peptides with incorporated heavy amino acids), and b) for efforts to purify and quantify peptides for absolute protein quantification.

JPT overcomes the traditional laborious and expensive purification and peptide content determination by attachment of a proprietary small chemical tag to the proteotypic peptide (proteotypic peptide + chemical tag, Figure 1).
Subsequent to the addition of a defined amount of tagged SpikeTide to the sample of interest, the digesting protease will release the desired proteolytic fragment from the SpikeTides in a defined 1:1 ratio, thus enabling the exact absolute quantification of the peptide. Alternatively to adding the SpikeTide to the sample of interest that is to be digested, the SpikeTide_TQL can also be proteolytically cleaved before addition to the sample.
The chemical tag is designed to be rapidly cleaved by trypsin that is commonly used for protein digestion. The concept of SpikeTides_TQL has been summarized[1] and was applied in a number of successful studies.[2]

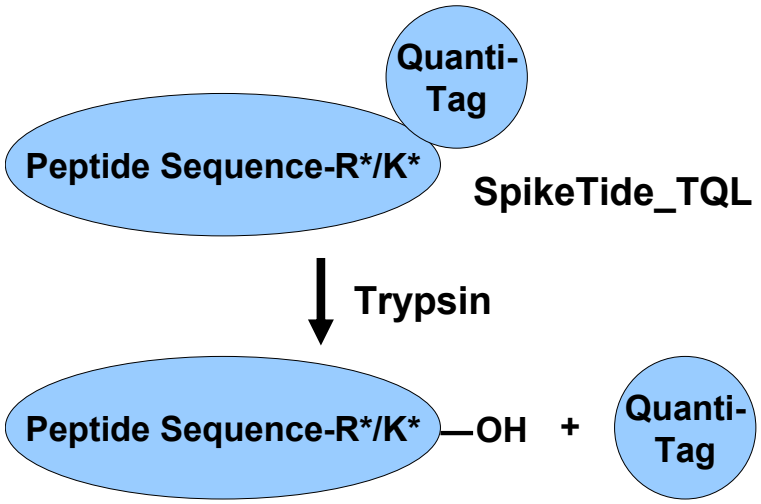

Figure 1: Release of a proteotypic peptide from the SpikeTide_TQL by proteolysis. Because the proteotypic peptide contains a Lys or Arg residue at the C-terminus, trypsin is used for processing the tagged SpikeTide_TQL.

An increasingly important discipline in proteomics is PTM proteomics – the measurement of posttranslationally modified (PTM) proteins. Despite recent improvements, PTM proteomics is still challenging.[3] Typical problems are a) low endogenous abundance, b) low ionization intensity, c) strong fragmentation, d) limited stability during proteomics workflows, and/or e) complex fragmentation spectra interpretation, especially regarding correct PTM site localization.
Providing defined synthetic PTM reference peptides is one way to support PTM proteomics and to help overcoming challenges. Accordingly, the recently launched ProteomeTools project[4] will incorporate reference spectra for hundred thousands of PTM modified peptides.

The PTM-kits and -peptides comprise peptides that can be utilized as PTM reference peptides. They consist of 142 differently modified peptides that were synthesized in a stable-isotope labeled (SIL) form, purified (purity >90%) and absolutely quantified with the help of the SpikeTides_TQL concept. An overview about the peptides in the collection can be found in the accompanying Excel file.

2 List of Components

Component	Quantity	Format
Sarstedt Vial(s)	Up to 142 SpikeTides_TQL labeled with stable isotopes (C-terminal Arg U-$^{13}C_6$;U-$^{15}N_4$ or Lys U-$^{13}C_6$;U-$^{15}N_2$). Tryptic digestion releases respective proteotypic peptides. Each peptide (5000 pmol / peptide) is supplied in a separate vial. **Aliquots: 1 x 5 nmol** quantified target peptide	Polypropylene vial (Sarstedt, #72.664.711)
CD-ROM	1	Microsoft Excel File

3 Storage

- All SpikeTides products should be stored at -20°C.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS!
PLEASE CONTACT JPT PEPTIDE TECHNOLOGIES´ TECHNICAL SERVICES FOR ASSISTANCE IF NECESSARY.

4 Additional Materials required

- Protease of adequate enzymatic activity which is able to cleave the peptide–tag peptide bond (like trypsin for peptides with Lys or Arg residues C-terminally of the proteotypic peptide): JPT recommends to use “Promega Sequencing Grade Modified Trypsin”.
- 0.1M Ammonium bicarbonate
- Dithiotreitol
- Iodoacetamide
- Formic acid

5 Experimental part

5.1 Experimental protocol

The tagged Spiketide peptides cannot be used as Spike-in controls for your assay solution without prior digestion to release the tryptic peptide.

1. Solubilize the peptides in a solution consisting of 80% of 0.1M ammonium bicarbonate and 20% acetonitrile.

As the peptides were synthesized with alkylated cysteine residues, steps 2 and 3 can be skipped

2. Add DTT to a final concentration of 12 mM in order to reduce all cysteine residues in your protein-containing sample. Incubate sample for 30 minutes at 32 °C.
3. Alkylate all Cys residues by adding iodoacetamide resulting in a final concentration of 40 mM. Incubate sample for 30 minutes at 25 °C in the dark. Dilute your solution by a factor of 3-4 with 0.1 M ammonium bicarbonate.
4. Add the tagged SpikeTides to your sample followed by the addition of protease for generation of proteotypic peptides. JPT recommends to use a weight-oriented dilution of 1/100-1/15 enzyme/substrate followed by an incubation of the sample for 16 h at 30°C (shaken) and rotation wheel for 4h at 30 °C.
5. Add formic acid to a final pH value of 3 to stop the enzymatic reaction.
6. Dry down the sample and resolubilize in 0.1 % formic acid (make sure that the pH value is acidic!).

	If protease concentration is too high, the enzyme might start cleaving amino acid bonds not typical for its proteolytic activity. Make sure to keep the enzyme concentration in the recommended range to get optimal results.

6 References

(1) Schnatbaum, K. et al. non-peer-reviewed application note in *Nature Methods* **2011**, 8.

(2) (a) Martínez-Morillo, E. et al. *J. Proteome. Res.* **2012**, *11*, 3880−3887. (b) Simicevic, J. et al. *Nat. Methods* **2013**, *10*, 570-576. (c) Kim J. S. et al. *J. Proteome. Res.* **2013**, *12*, 2582−2596. (d) Martínez-Aguilar, J. et al. *J. Proteome. Res.* **2013**, *12*, 3679−3688. (e) Saito, M. A. et al. *Science* **2014**, *345*, 1173-1177. (f) Martínez-Aguilar, J. et al. *BMC Cancer* ***2015***, *15*, 1-14. (g) Korbakis, D. et al. *Mol. Cell Proteomics* **2015**, *14*, 1517-1526. (h) Saito, M. A. et al. Proteomics **2015**, *15*, 3521-3531.

(3) 1) (a) Olsen, J. V. et al. *Mol. Cell. Proteomics* **2013**, *12*, 3444-2452. (b) Doll, S. et al. *ACS Chem Biol.* **2015**, *10(1)*, 63-71.

(4) Zolg, D. P. et al. *Nat. Methods* **2017**, *14(3)*, 259-262.