User Manual

SpikeTides Set Histone – heavy – quantified

Collection of stable isotope labeled proteotypic peptides for absolute quantification of histone H3 including acetylated and methylated lysines

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Please read the entire manual before starting your experiments!
Carefully note the handling and storage conditions.
For research use only.

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1. Introduction

Histones are subject to post-translational modifications (epigenetic marks) that influence chromatin structures. The histone code hypothesis suggests that combinations of these modifications play a key role in the regulation of gene expression and thereby are important for health and disease. The examination of the histone code as well as the identification of histone based biomarkers is dependent on the availability of methods for the efficient multiplexed detection and quantification of the respective (modified) protein. Therefore, a kit was developed that covers the most important and most well-characterized lysine modifications of histone H3.

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.

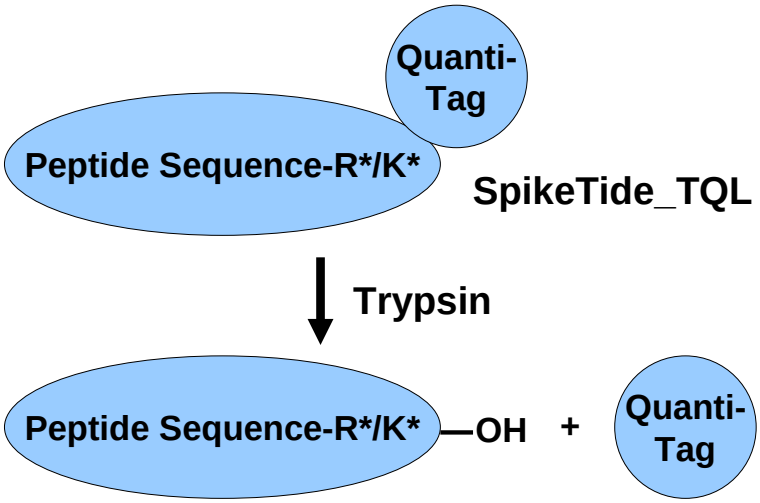

Figure 1: Release of a proteotypic peptide from the SpikeTide_TQL by proteolysis. Because the proteotypic peptide contains a Lys or Arg residue at the C-terminus, trypsin is used for processing the tagged SpikeTide_TQL.

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).

2. SpikeTides Set Histones – heavy -quantified

Due to the high promise of modified histones for the stratification of diseases like cancer, the concept of SpikeTides_TQL was used for the development of a kit of absolutely quantified peptides for multiplexed absolute quantification of these epigenetic marks.
Eight proteotypic peptides of unmodified histone H3 together with proteotypic peptides displaying acetylation and different methylation states of nine lysine residues were selected based on literature. The peptides were synthesized and absolutely quantified with the help of the SpikeTides_TQL concept.
In summary, a collection of a large number of absolutely quantified stable isotope labeled peptides is presented. The kit enables the quantitative detection of histone H3 including the major acetylation and methylation states from complex protein mixtures in a concentration range spanning several orders of magnitudes in biological fluids and tissues to support diagnosis and stratification of cancer and other applications.

2.1 Storage & Handling

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

2.2 List of Components

Component	Quantity	Format
96 tube rack	37 SpikeTides_TQL Labeled with stable isotopes: C-terminal Arg U-13C6;U-15N4 or Lys U-13C6;U-15N2 Tryptic digestion releases respective proteotypic peptides. Aliquots: **1 x 1nmol** quantified target peptide	96 well micro-tube rack system (HJ-Bioanalytik, 1.4mL tubes)
Product documentation	1	Microsoft Excel File

2.3 Additional Material Required

- Protease of adequate enzymatic activity
    - 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

2.4 Tube Rack Layout

The product documentation provided by email or available for download contains all information needed for easy allocation of peptide sequence to the Micronics rack tube number. The numbering starts with well A1 in the upper left corner, counting the first 12 peptides up to well A12. Peptide 13 is deposited in well B1 and so on. All other columns and rows are filled likewise.
The Micronics rack tubes are delivered with a yellow lid, keeping environmental air and humidity out of the tubes.

3. Experimental Protocol

The following procedures are provided only as guidelines. The optimal experimental conditions will vary depending on the sample and instruments used.

The tagged SpikeTides 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.

4. References

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

(2) (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.

5. Contact Us

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6. Product Use & Liability

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