Protocol

SpikeTides™

Peptides for relative and absolute quantification in SRM and MRM Assays

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1 Introduction

Peptide quantification is a prerequisite in many areas of proteomics. One approach for detection and quantitative analysis of targeted proteins in complex mixtures relates to the use of tandem mass spectrometry to monitor one or more proteotypic peptide(s) from one protein of interest by a selected reaction monitoring (SRM) assay or by parallel analysis of many proteotypic peptides by a multiple reaction monitoring (MRM) assay. Relative and absolute quantification is performed by the use of stable isotope-labeled proteotypic peptides as internal standards. For absolute quantification these standards have to be purified to 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 this situation by using a high-throughput peptide synthesis platform that yields small scale, unpurified light and heavy labeled peptides at a fraction of the costs of standard solid phase approaches. In addition laborious and expensive purification and peptide content determination is avoided by attachment of a proprietary small chemical tag to the proteotypic peptide (proteotypic peptide + chemical tag). The chemical tag is designed to be cleaved by proteases commonly used for protein digestion and can be applied for determining the absolute SpikeTide amount.

SpikeTides are categorized in following groups of peptide libraries:

SpikeTides: small scale, unpurified proteotypic peptides with C-terminal lysine or arginine.

SpikeTides L: SpikeTides labeled with stable isotopes (C-terminal Arg U-13C6;U-15N4 or Lys U-13C6;U-15N2). Amount: 50nmol/total peptide.

SpikeTides TQ: SpikeTides provided with information on absolute quantity for each peptide using JPT's Quanti-Tag. SpikeTides TQ need to undergo tryptic digestion to release respective proteotypic peptide. Amount: 5 x 1nmol quantified target peptide.

SpikeTides TQL: SpikeTides labeled with stable isotopes (C-terminal Arg U-13C6;U-15N4 or Lys U-13C6;U-15N2) provided with information on absolute quantity for each peptide. SpikeTides TQL need to undergo tryptic digestion to release respective proteotypic peptide. Amount: 5 x 1nmol quantified target peptide.

Once a sample is spiked with the appropriate SpikeTides TQ or SpikeTides TQL (or a corresponding mixture) the used protease (mostly trypsin) will cleave the peptide/tag-bond releasing the desired proteotypic peptide and the JPT-tag stoichometrically in a one to one ratio. Alternatively, the SpikeTides TQ or SpikeTides TQL can be proteolytically cleaved before addition to the sample of interest.

2 List of Components

Component	**Format**
Download-Link / Email by Customer Support	Microsoft Excel file

Depending on product specifications:

Product	**Component**	**Quantity**	**Format ***
SpikeTides SpikeTides L	Microtiter plate	depends on number of peptides and required MTP layout; please refer to section 5.2 for details	96 well format (Greiner Bio-one, PP, #650201)
SpikeTides TQ SpikeTides TQL SpikeTides Maxi TQ SpikeTides Maxi TQL	Micronics rack	depends on number of peptides and required rack layout **Aliquots: 5 x 1nmol** per peptide, other aliquotation formats upon request	96 well format (Micronics Roborack 1.4mL tubes)
SpikeTides Maxi SpikeTides Maxi L	Cryo-tubes or Microtiter plate	0.5mg / peptide	Stor-It Cryo tubes (Nunc/VWR 391-8342) 96 well format (Greiner Bio-one, PP, #650201)
SpikeMix SpikeMix L	Vials	10 individual Vials per Pool Approx. 0.2 nmol/peptide/aliquot	Vials (Sarstedt, 2mL PP, 72.664.711)

* Format may vary depending on ordered amounts

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

- For SpikeTides TQ and SpikeTides TQL, 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)
    - for tryptic digestions JPT recommends to use
      Promega Sequencing Grade Modified Trypsin
- 0.1M ammonium bicarbonate
- dithiotreitol
- iodoacetamide
- formic acid

5 General considerations

5.1 Experimental basics

SpikeTides represent peptide derivatives resulting from proteolytic fragments of proteins. Each SpikeTide (TQ and TQL) consists of the proteolytic fragment fused to the proprietary JPT-tag. Subsequent to addition of 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 amount enabling quantification of peptides if heavily labeled amino acid residues were used and if the amount of the added SpikeTide was determined exactly (see figure 1). If quantified SpikeTides (SpikeTide TQ or SpikeTides TQL) are used absolute quantification within one analysis is possible.

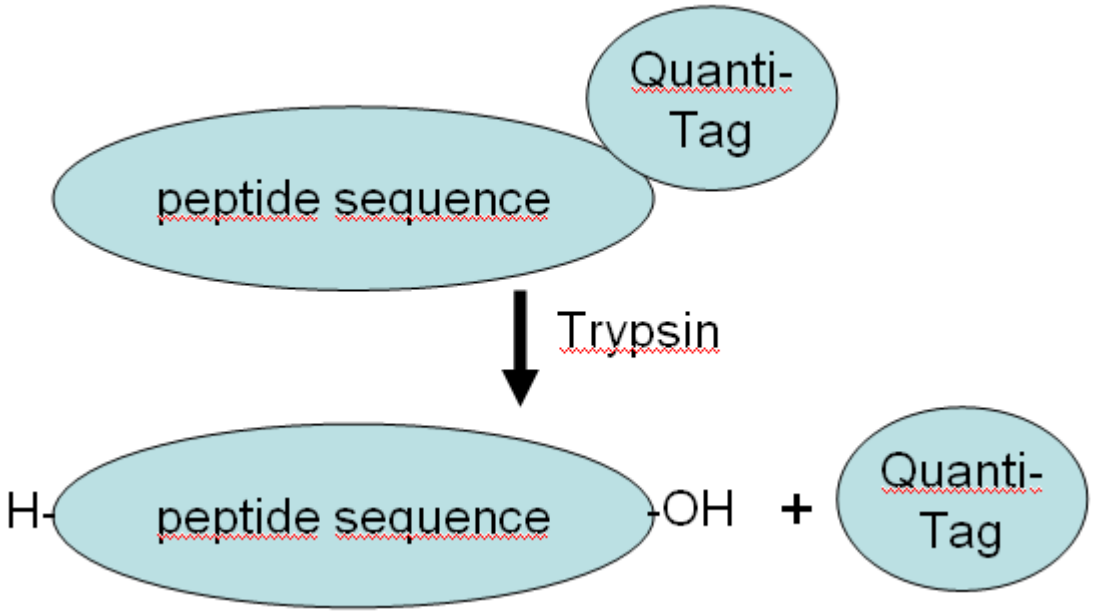

Figure 1: Release proteotypic peptide from the SpikeTide by proteolysis. If proteotypic peptide contains a Lys or Arg residue at the C-terminus, trypsin should be used for processing the tagged SpikeTide

5.2 Microtiter Plate Layout

The data-files provided with the SpikeTides contains all information needed for easy allocation of peptide sequence to both, microtiter plates and wells / micronic rack and tube number or pool aliquot. For microtiterplates / micronic racks, by default 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 microtiter plate is delivered with a lid as well as an additional sealing mat, keeping environmental air and humidity out of the individual wells. Please see figure 2 for details:

Figure 2: Left: Microtiter-Plate delivery format, lidded and sealed; Right: individual components: Lid, seal and microtiter-Plate

Make sure to remove the sealing mat before adding solution to the microtiter plate wells !

6 Experimental protocols

6.1 SpikeTides and SpikeTides L

The non-tagged Spiketide peptides can be directly used as Spike-in controls for your assay solution.

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

If peptides were ordered with alkylated cysteine residues, steps 2 and 3 can be skipped

2. Add DTT to a final concentration of 12mM in order to reduce all cysteine residues in your SpikeTide- sample. Incubate sample for 30 minutes at 32°C
3. Alkylate all Cys-residues by adding iodoacetamide resulting in a final concentration of 40mM. Incubate sample for 30minutes at 25°C in the dark
4. Dilute your solution by factor 3-4 with 0.1M ammonium bicarbonate
5. Add non tagged SpikeTides to your sample followed by the addition of protease. If trypsin is used as protease JPT recommends to use a weight-oriented dilution of 1/100 enzyme/substrate followed by an incubation of the sample for 5h at 37°C
6. Add formic acid to a final pH value of 3 to stop the enzymatic reaction
7. Dry down the sample and resolubilize in 0.1% formic acid (make sure that the pH value is acidic!)

6.2 Tagged SpikeTides TQ and TQL

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

If peptides were ordered with alkylated cysteine residues, steps 2 and 3 can be skipped

2. Add DTT to a final concentration of 12mM 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 40mM. Incubate sample for 30minutes at 25°C in the dark Dilute your solution by factor 3-4 with 0.1M ammonium bicarbonate
4. Add tagged SpikeTides to your sample followed by the addition of protease for generation of proteotypic peptides. If trypsin is used as protease JPT recommends to use a weight-oriented dilution of 1/100 enzyme/substrate followed by an incubation of the sample for 5h at 37°C. The Spiketide peptides will be digested in parallel to your protein sample.
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 values to get optimal results.