Protocol

SpikeTides™ Sets
SpikeTides™ Sets– heavy
SpikeMix™
SpikeMix™– heavy

Peptide Sets for relative quantification of Proteins in Mass Spectrometry Based Assays

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1 Introduction

Quantitative proteomics plays an intergral part in both discovery and targeted proteomic analyses. One common approach is to combine stable isotopically labeled peptides as internal standards with liquid chromatography mass spectrometry (LC-MS) to identify and quantitate complex proteins mixtures from cells, tissues or organisms. Utilizing stable isotope-labeled proteotypic peptides in conjunction with a targeted proteomic assay (SRM/MRM/PRM) can provide relative quantification analysis of many proteotypic peptides in a single LC-MS run. In addition, standards can be used for both retention time calibration and data normalization in label-free and data-independent assays.

JPT´s unique high-throughput peptide synthesis platform yields small scale, unpurified light and heavy labeled peptides at a fraction of the costs of standard solid phase approaches. Based on this technology, JPT has developed a number of pre-manufactured SpikeMixes™ and SpikeTides™ Sets for rapid quantitative mass spectrometric assay development and relative protein quantification. The peptides of each set are selected to represent proteotypic peptides for a carefully chosen group of proteins. This enables answering complex questions at an unparalleled speed and cost effectiveness.

We offer SpikeTides™ Sets and SpikeMix™ pools as light or stable isotope-labeled (heavy) peptides:

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).

Quantified SpikeTides™_TQL for absolute quantification of proteins are also available from JPT upon request.

The described groups of SpikeTides™ can be prepared in two different formats:

- Individual peptides in microtiter plates or micronics tubes (SpikeTides™ Sets).
- Mixtures (pools) of peptides in polypropylene vials (SpikeMix™).

2 List of Components

Component	Quantity	Format
CD-ROM	1	Microsoft Excel file

Depending on product specifications:

SpikeTides™ Sets: Microtiter plate(s) (Greiner Bio-one, PP, #650201)

SpikeMix™: Polypropylene vial (Sarstedt, #72.664.711)

The data CD-ROM provided contains all required information, esp. peptide sequences and their annotation to associated proteins.

For the SpikeTides™ Sets, the allocation of the peptide sequences to the microtiter plate wells is also included in the data CD-ROM. 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. Row H is left blank for optional controls etc.

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 1 for details:

Figure 1: 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 !

Please note that all cysteines of the peptide set have been alkylated with iodoacetamide to maximize compatibility with typical proteomic workflows.

Please note that due to the nature of the targeted proteins, several peptides might be very hydrophilic. Please make sure to adapt your analysis workflow and instrument setup accordingly.

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

- 0.1M ammonium bicarbonate
- Acetonitrile
- Dithiotreitol (DTT)
- Iodoacetamide
- Formic acid

5 Experimental protocols

5.1 Standard Protocol

The Spiketides™ peptides can be used directly as spike-in controls for your assay solution.

1. Solubilize the SpikeTides™ peptides in a solution consisting of 80% of 0.1M ammonium bicarbonate and 20% acetonitrile.
2. Add the SpikeTides™ peptides to your sample dissolved in 0.1M ammonium bicarbonate.
3. Add DTT to a final concentration of 12 mM in order to reduce all cysteine residues in your sample. Incubate sample for 30 minutes at 32°C.
4. 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.
5. Add your protease (e.g. trypsin in a 1/100-1/15 enzyme/substrate ratio) and incubate at an appropriate temperature (e.g. RT, 16 hours).
6. Add formic acid to a final pH value of ≤ 3 to stop the enzymatic reaction.
7. Optionally dry down the sample and resolubilize in 0.1% formic acid (make sure that the pH value is acidic!).
8. Perform LC-MS analysis.

5.2 Special Protocol for SpikeMix™ – ABRF (cross-species standard)

Following instructions from ABRF we recommend the following procedures for handling the SpikeMix™ – ABRF (cross-species standard):

If adding prior to enzymatic digestion

1) Dissolve and vortex the mixture of peptides in 70% formic acid.
2) Dilute with 0.1M ammonium bicarbonate to a final ratio of 1:6 of 70% formic acid/ 0.1 M ammonium bicarbonate.
3) Add the SpikeMix™ to your sample dissolved in 0.1M ammonium bicarbonate.
4) Proceed with reduction, alkylation, and enzymatic digestion as described above.

If adding the peptides prior to LC-MS analysis

1) Dissolve and vortex the mixture of peptides in 70% formic acid.
2) Dilute with 0.1% TFA in water to a final ratio of 1:6 of 70% formic acid/ 0.1% TFA in water.
3) Add the SpikeMix™ to your digestion mixture and proceed with a standard LC-MS protocol.

5.3 Special Protocol for SpikeMix™ – CEF (ext) Pool

For characterization of T-cell stimulating peptides, the CEF positive control pool can be applied. Since these peptides are not derived from proteotypic cleavage, no digestion step is necessary. We recommend the following protocol:

1) Dissolve and vortex the mixture of peptides in a solution consisting of 80% water and 20% acetonitrile.
2) Add the SpikeMix™ to your mixture of eluted epitope peptides and proceed with a standard LC-MS protocol.