An Easy to Use Peptide Kit to Monitor Efficiency & Reproducibility of Tryptic Sample Preparation

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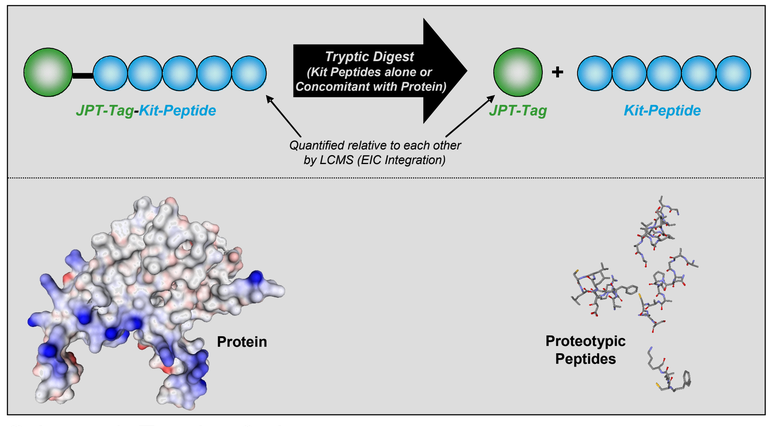
Introduction

The complete and reproducible tryptic digestion of proteins in biological samples is an important step in MS-based proteomic workflows. Incomplete digestion will impair the qualitative and quantitative results of such experiments. Different protocols for tryptic digestion have been described and available trypsins vary in quality. Therefore, an easy to use test kit to monitor the efficiency and reproducibility of tryptic digestion is highly desirable.

Principle

The goal of the development was the design and generation of a peptide kit consisting of a mixture of peptides labeled with tryptic tags (SpikeTides™) displaying defined but variable cleavage kinetics.
The principle of the tagging and cleavage process with SpikeTides™ is shown in scheme 1.



Scheme 1: Tagging & cleavage process.

A) Independent digestion of the kit peptides allows the evaluation of different trypsin lots and digestion conditions.
B) When kit peptides are digested along with proteins, the degree of cleavage of the tagged kit peptides shows protein cleavage efficiency.

Design Criteria for the Peptides:
- Different resistance to tryptic cleavage
- Unique MS-signals and sufficient LC-MS separation for peptides and cleavage products (JPT-Tag & peptide)
- No occurrence in native proteins
- Retention times of cleaved peptides span a wide RT-range allowing correlation with SSRT values

Methods

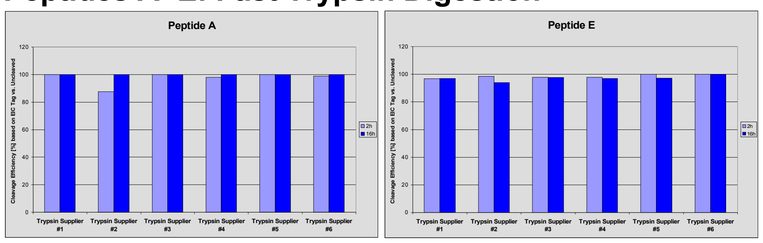
The kit peptide mixture (table 1) was subjected to digestion with trypsin from different suppliers

A) Trypsin/substrate ratio 1:100, 2h
B) Trypsin/substrate ratio 1:50, 16h

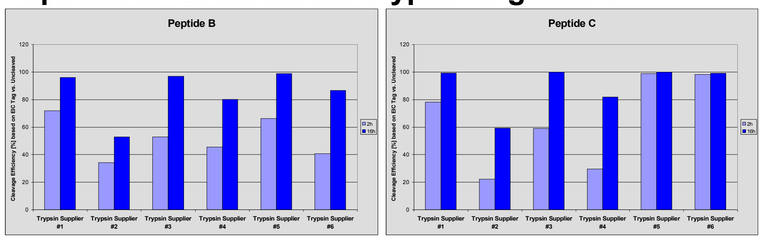
Readout: Relative quantification of cleavage product (JPT-Tag) vs. starting material (JPT-Tag-Kit-Peptide) via EIC integration.

Results

Peptides A+E: Fast Trypsin Digestion



Peptides B+C: Moderate Trypsin Digestion



Peptide D: Slow Trypsin Digestion

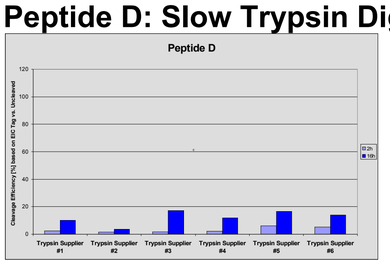


Figure 1: Cleavage efficiency (percent cleavage) of peptides under different digestion conditions.

Conclusion: The peptide set is well suited to determine differences in digestion conditions and between trypsin lots from various suppliers.

Peptide No.	Sequence
A	JPT-Tag1-GSGSGGHGGR
B	JPT-Tag2-LDQSENPEWK
C	JPT-Tag3-ELLQESAILR
D	JPT-Tag4-PGLAEIEFWR
E	JPT-Tag5-TAEADGGALR

Table 1: Kit peptides.

Peptide Set spiked into Protein Digest: "*in situ*" Evaluation of Trypsin Cleavage Efficiency

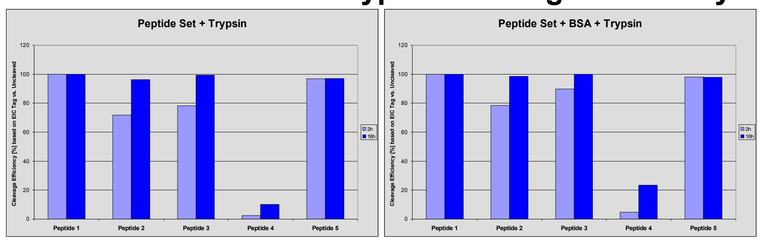


Figure 2: Cleavage efficiency of peptides without/with BSA.

Conclusion: Protein and peptide set digestion can be performed in the same vial without mutual interference.

Standards for Retention Time Calibration

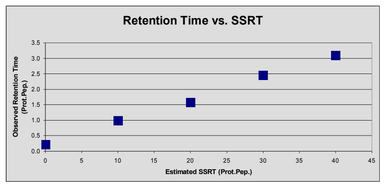


Figure 3: Retention time of cleavage products (proteotypic peptides) vs. estimated SSRT value.

Conclusion: The peptide set can be used to correlate retention time to SSRT value.

Summary

- The new peptide kit enables accurate *in-situ* determination of trypsin cleavage efficiency and reproducibility
- The digestion is compatible with routine sample digestion workflows
- Analysis can be performed using standard LC-MS protocols
- Additionally, digested peptides can be used as retention time calibration standards
- The absolute quantification of cleavage products in the samples will be achieved with heavily labeled tags
- The peptide set will be commercially available in due course

Easy to use kit for efficient monitoring of trypsin digestion.

References

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Picotti, P., Bodenmiller, B., Mueller, L.N., Domon, B., & Aebersold, R. *Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics.* Cell **138**, 795–806 (2009).
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