SpikeTides™ - Peptide Standards for Cancer Biomarker Screening and Monitoring of Tryptic Digestion

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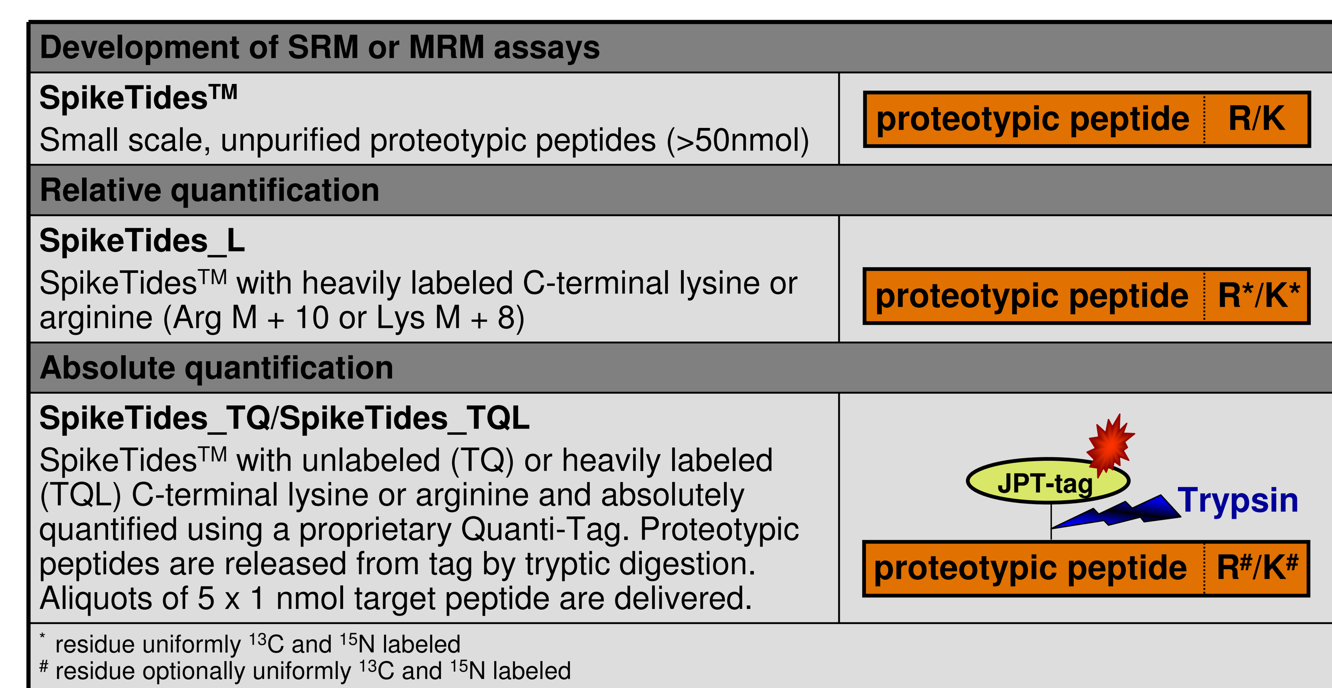
Introduction

SpikeTides™ have recently been described as a low cost peptide source for efficient development of MRM assays. Furthermore, SpikeTides™ have been used successfully as heavily labelled, internal peptide standards for absolute and relative quantification of protein expression levels[1,2] (Table 1).
In order to further exploit the potential of SpikeTides™, two new applications have been targeted.



Development of SRM or MRM assays	
SpikeTides™ Small scale, unpurified proteotypic peptides (>50nmol)	proteotypic peptide R/K
Relative quantification	
SpikeTides_L SpikeTides™ with heavily labeled C-terminal lysine or arginine (Arg M + 10 or Lys M + 8)	proteotypic peptide R*/K*
Absolute quantification	
SpikeTides_TQ/SpikeTides_TQL SpikeTides™ with unlabeled (TQ) or heavily labeled (TQL) C-terminal lysine or arginine and absolutely quantified using a proprietary Quanti-Tag. Proteotypic peptides are released from tag by tryptic digestion. Aliquots of 5 x 1 nmol target peptide are delivered.	JPT-tag Trypsin proteotypic peptide R#/K#

* residue uniformly ^{13}C and ^{15}N labeled
# residue optionally uniformly ^{13}C and ^{15}N labeled

Table 1: Variants of SpikeTides™.

TAA SpikeTides™ Sets

Tumor associated antigens (TAAs) are highly promising as potential cancer biomarkers and targets for cancer vaccines. However, the full wealth of TAA research is expected to be uncovered by the parallel quantification of TAAs, which currently is hampered by the high costs of reference standards.
We designed a SpikeTides™ peptide set containing 252 light or heavily labeled peptides. The peptides were derived from relevant tumor associated antigens (TAAs, Table 2) deducted from an NCI prioritization approach[3] and available SRM assay conditions[4]. The peptide set contains 1-6 (on average 4) peptides per protein.

No.	Protein	No.	Protein	No.	Protein	No.	Protein	No.	Protein	No.	Protein
1	WT1	12	MAR1	23	PPAP	34	RHOC	45	RGS5	56	CD276
2	MUC1	13	RASN	24	BIRC7	35	TYRP2	46	SART3	57	LGMN
3	EGFR	14	PMEL	25	FETA	36	MSLN	47	CAH9	58	TIE2
4	ERBB2	15	PRTN3	26	EPCAM	37	PSCA	48	PAX5	59	GAGC1
5	MAGA3	16	ABL1	27	ERG	38	MAGA1	49	ACRBP	60	VGFR2
6	MAGA2	17	BCR	28	TMPS2	39	CP1B1	50	SP17	61	PRM2
7	MAGC1	18	TYRO	29	PAX3	40	PLAC1	51	LCK	62	SEPR
8	P53	19	BIRC5	30	ALK	41	CTCFL	52	CSPG4	63	PGFRB
9	CTG1B	20	KLK3	31	ANDR	42	ETV6	53	AKAP4	64	FOSL1
10	FOLH1	21	TERT	32	CCNB1	43	RUNX1	54	SSX2		
11	CEAM5	22	EPHA2	33	MYCN	44	AN30A	55	GAGD2		

Table 2: TAAs covered by the TAA SpikeTides™ set.

Experiment:
Peptide Identification of both the heavy and light version of all 252 TAA SpikeTides™ set were confirmed using a 5600 TripleTOF mass spectrometer. Serial dilutions of the light peptides showed a majority of peptides behaved as expected with a linear response. The resulting spectral library can be used to generate a targeted proteomics assay for estimated concentration analysis.

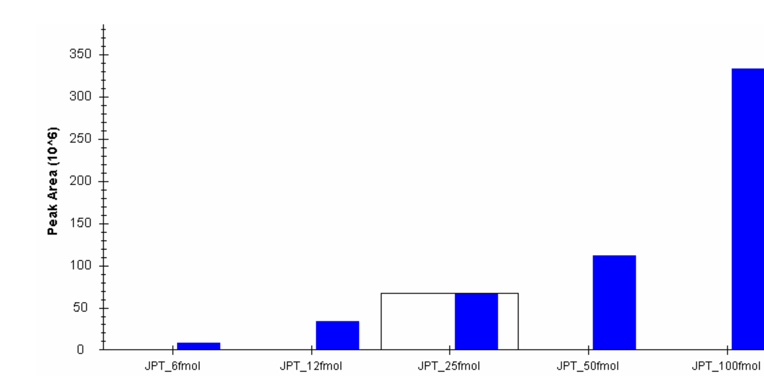


Figure 1.
Serial Dilution of the QGGFLGLSNIK Peptides

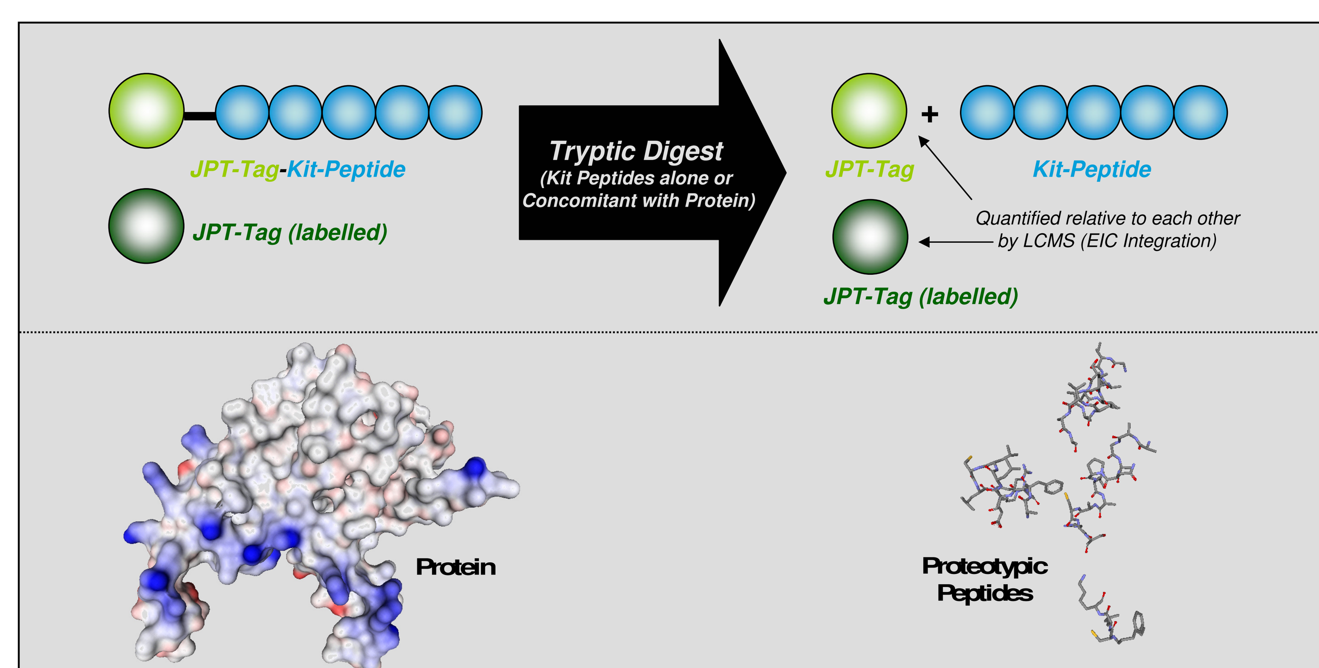
Summary:
- The TAA SpikeTides™ set is easy to use and enables – for the first time – the cost-efficient multiplexed detection and relative quantification of TAA expression levels in biological samples

TAA SpikeTides™ sets – Easy to use peptide sets for parallel monitoring of TAAs

TrypCheck Kit

Since reliable digestion is essential for all MS based proteomics approaches, the development of robust and validated protocols is crucial[2]. This was addressed by the development of a ready-to-use kit – TrypCheck – that enables monitoring of both, the efficiency and the reproducibility of tryptic digests of biological samples.

Principle of the tagging and cleavage process:



Scheme 1: Tagging & cleavage process. Top: Independent digestion of the kit peptides allows the evaluation of different trypsin lots and digestion conditions. Bottom: When kit peptides are digested along with proteins, the degree of cleavage of the tagged kit peptides parallels protein cleavage efficiency.

TrypCheck kit peptides:

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

Table 3: Kit peptides.

Experiment:
Digestion of the TrypCheck kit with Trypsin under varying conditions.

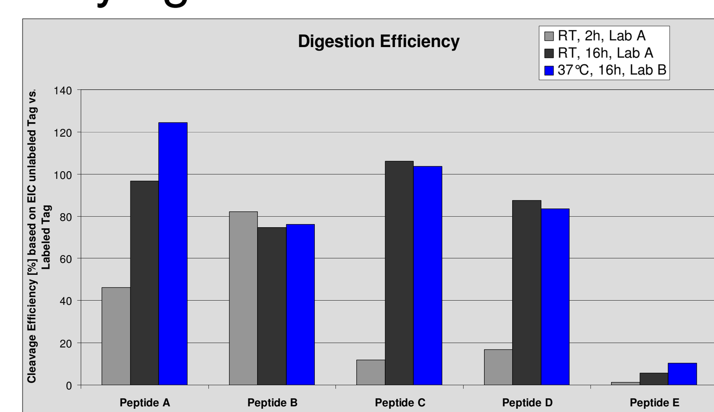


Figure 2: Cleavage efficiency (percent cleavage) of peptides under different digestion conditions.

Figure 2 shows that the peptide set is well suited to determine differences in digestion conditions. While after incubation for 2 hours the digestion is incomplete, longer incubation leads to complete digestion of peptides A-D. Peptide E (control peptide) is not cleaved.

Summary:
- The TrypCheck 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 and for estimating contamination of Trypsin by Chymotrypsin (data not shown)

TrypCheck kit – An easy to use kit for efficient monitoring of trypsin digestion

References

(1) (a) Picotti, P. et al., High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nat. Methods*, **2010**, *7*, 43-46.
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(3) Cheever, M. A. et al., The Prioritization of Cancer Antigens: A National Cancer Institute Pilot Project for the Acceleration of Translational Research. Clin. Cancer Res. **2009**, *15*, 5323-5337.
(4) www.srmatlas.org, ISB.

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