Protocol

TrypCheck Kit_Fluorescence

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

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1 Introduction

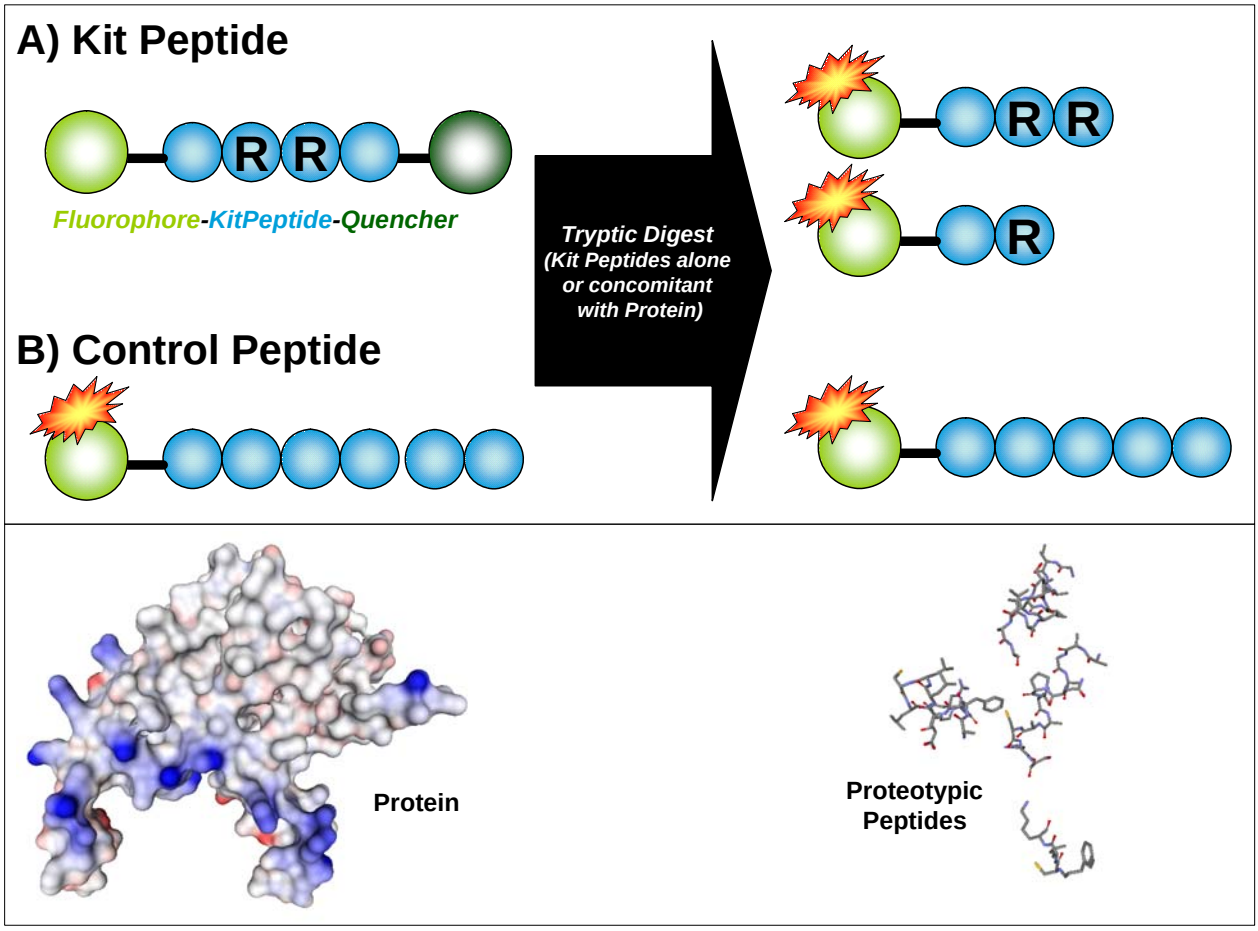
The complete and reproducible tryptic digestion of proteins in biological samples is one of the most crucial steps in MS-based proteomic workflows. Incomplete digestion will impair the qualitative and quantitative results of such experiments. The situation is complicated by the fact that different protocols for tryptic digestion have been described and available trypsins vary in quality.[1,2]

To rapidly assess tryptic digestion efficacy, the easy to use "TrypCheck Kit_Fluorescence" has been developed. The kit makes use of rapidly and easily measured fluorescence readout. For more in-depth analysis of tryptic digestion efficacy, JPT developed the "TrypCheck Kit", which is based on more accurate readout by MS.

Determination of Digestion Efficacy

The principle of the "TrypCheck Kit_Fluorescence" is based on the FRET concept and is shown in Scheme 1 (for one of the kit peptides). A peptide bearing one or more Arg or Lys in the center of its sequence (light blue) covalently connects a fluorophore (Abz, light green) and its respective quencher (Nitrotyrosine, dark blue). Upon tryptic digestion at the R/K site the quencher is released from the peptide, giving rise to the unquenched "free" fluorophore. The amount of the released fluorophore directly correlates with the cleavage efficiency of the digestion.

To quantify the released fluorophore, a control peptide (Scheme 1B) has been included in the kit. It is provided in an amout that correnspponds to a 1:1 molar ratio compared to the other peptides. Therefore, the ratio of the fluorescence signal from the digest of the kit peptide, divided by the signal from the control peptide, directly gives the percentage of cleavage of the kit peptide.



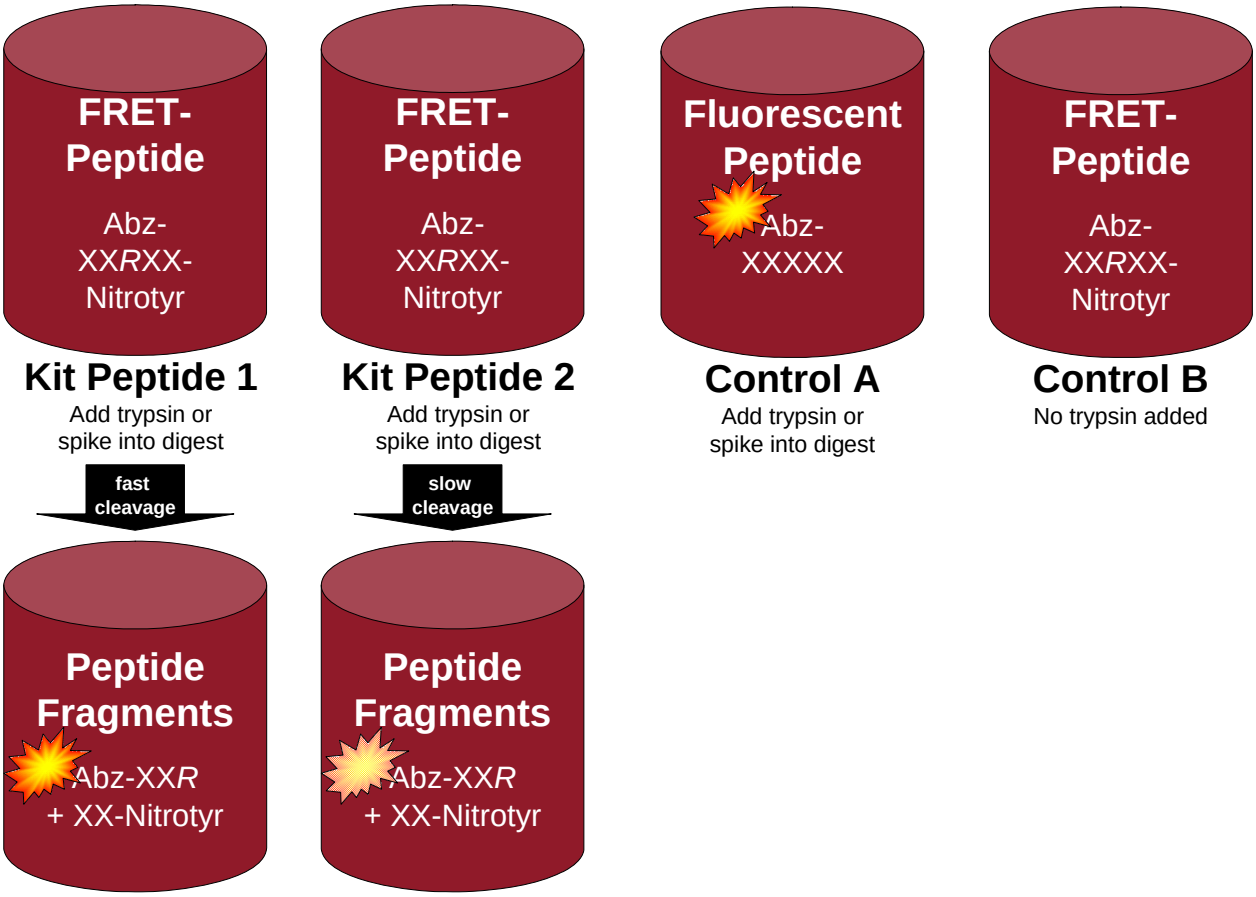
Scheme 1: Tagging & cleavage process (exemplarily for one kit peptide).

The “TrypCheck Kit_Fluorescence” is applied mainly for two different applications:

A) Independent digestion of the kit peptides allows the rapid evaluation of trypsin digestion (Scheme 1, top).

B) When the kit peptides are digested along with proteins, the degree of cleavage of the tagged kit peptides can be used as a measure of protein cleavage efficiency (Scheme 1, top and bottom).

The “TrypCheck Kit_Fluorescence” contains two different peptides accompanied by two controls. The peptides were designed to have different resistance to tryptic cleavage: one peptide (peptide 1) is very rapidly cleaved by Trypsin, while the other peptide (peptide 2) is more resistant to tryptic cleavage.



Results of a Typical Digestion Experiment

The kit peptides were subjected to digestion with trypsin under the following conditions: Trypsin/substrate ratio 1:50, RT (room temperature). Figure 2 shows that the peptide set is well suited to follow the course of the digestion. While peptide 1 (orange lines) is fully cleaved already after 30 minutes, peptide 2 requires about 5 hours until full cleavage.

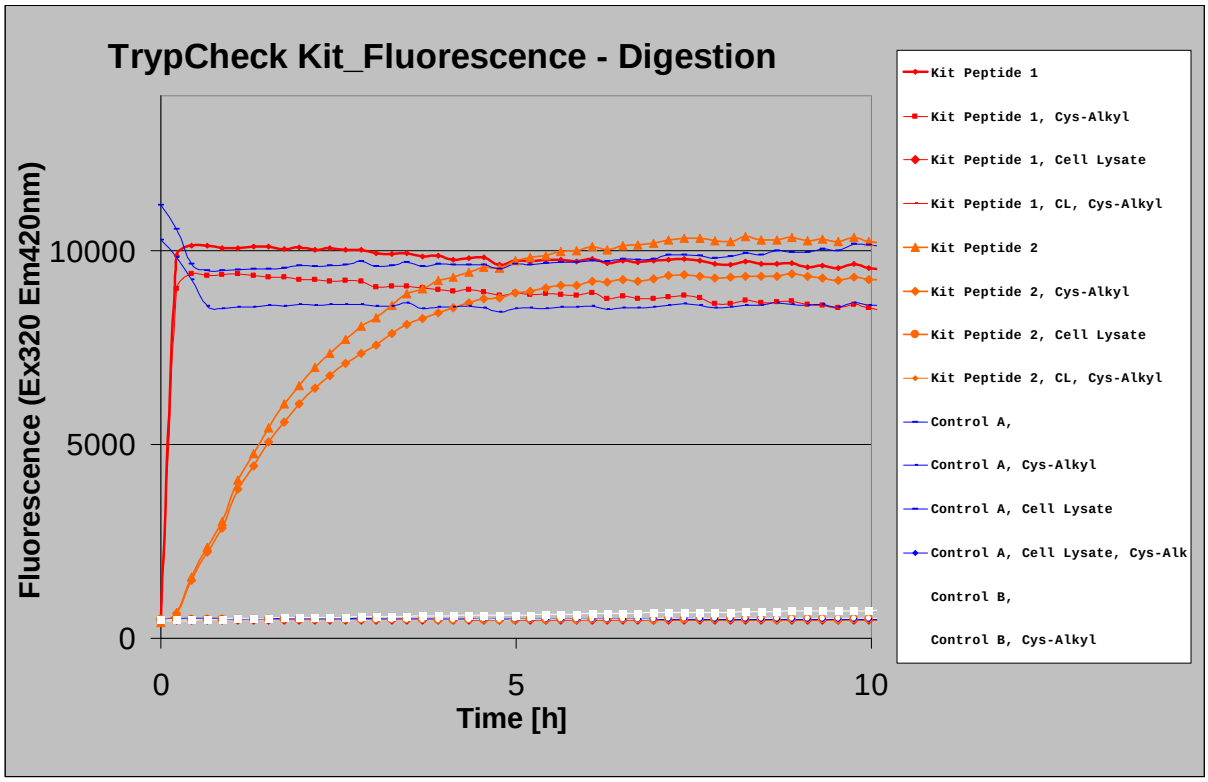


Figure 2: Cleavage of the kit peptides by trypsin followed by fluorescence readout (15 min intervals, 10 hours).

Summary

The “TrypCheck Kit_Fluorescence” enables rapid fluorescence-based monitoring of trypsin activity.

References

(1) Glatter, T., Ludwig, C., Ahrné, E., Aebersold, R., Heck, A. J. R., Schmidt, A. Large-Scale Quantitative Assessment of Different In-Solution Protein Digestion Protocols Reveals Superior Cleavage Efficiency of Tandem Lys-C/Trypsin Proteolysis over Trypsin Digestion. *J. Proteome Res.* **2012**, *11*, 5145-5156.

(2) Burkhart, J. M., Schumbrutzki, C., Wortelkamp, S., Sickmann, A., Zahedi, R. P., Systematic and quantitative comparison of digest efficiency and specificity reveals the impact of trypsin quality on MS-based proteomics *J. Proteomics* **2012**, *75(4)*, 1454-1462.

2 List of Components

Polypropylene vials with the following compounds:

Name	Sequence	Amount [nmol]	Amount [µg]	Ease of Cleavage by Trypsin
Kit Peptide 1	Abz-ARRA-Nitrotyr-NH_2	200	160	++
Kit Peptide 2	Abz-AVKD-Nitrotyr-NH_2	200	152	+
Control A	Abz-AR-OH	200	73	n/a
Control B	Abz-ARRA-Nitrotyr-NH_2	200	160	n/a

3 Storage

- The “TrypCheck Kit_Fluorescence” should be stored at -20°C.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS!

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4 Experimental Protocols

1. Solubilize the kit peptides in a solution of 0.1 M aqueous ammonium bicarbonate and 0-20% acetonitrile (each in 100 µL; for complete dissolution apply vortexer for at least 20 seconds). Use 10 µL of each peptide solution for step 2.
2. Add an aliquot of a solution of activated trypsin (the trypsin solution for which the digestion efficacy is to be monitored; e.g. 0.32 µg of activated trypsin for an enzyme substrate ratio of 1:50 for kit peptide 1) according to the following table. The trypsin solution might also contain a protein that is to be digested.

Name	Sequence	Add Trypsin (yes/no)
Kit Peptide 1	Abz-ARRA-Nitrotyr-NH_2	yes
Kit Peptide 2	Abz-AVKD-Nitrotyr-NH_2	yes
Control A	Abz-AR-OH	yes
Control B	Abz-ARRA-Nitrotyr-NH_2	**no**

Standard tryptic workflows usually employ DTT/TCEP and Iodoacetamide for reduction and alkylation of cysteine residues (steps 3 and 4 in this protocol). These steps (3 and 4) are not necessary for the performance of the kit, as the kit does not contain cysteines. However, it is not detrimental for the performance of the kit to perform a reduction and alkylation step (steps 3 and 4).

3. Add TCEP to a final concentration of 5 mM in order to reduce all cysteine residues in your protein-containing sample. Incubate sample for 60 minutes at 37°C.
4. Alkylate all Cys-residues by adding iodoacetamide resulting in a final concentration of 0.5 M. Incubate sample for 30 minutes at 25°C in the dark.

5. Analyze samples by fluorescence (Excitation 320 nm, Emission 420 nm).

5 Readout

Fill out the blank fields in the following table.

Fluorescence Signal	Fluorescence Signal	Fluorescence Signal	Calculate Digestion Efficacy [%]
Kit Peptide 1 (X1)	Control A (CA)	Control B (CB)	(X1-CB) / (CA-CB) * 100
Kit Peptide 2 (X2)	Control A (CA)	Control B (CB)	(X2-CB) / (CA-CB) * 100