

User Manual

TrypCheck Kit_Fluorescence

Easy-to-use kit to estimate efficiency & reproducibility of tryptic sample preparation

Table of Contents

1. Introduction	1
2. TrypCheck Kit_Fluorescence	2
2.1 Determination of Digestion Efficacy	2
2.2 Results of a Typical Digestion Experiment	3
2.3 List of Components	4
2.4 Storage	4
3. Experimental Protocols	5
3.1. Tryptic Digestion.....	5
3.2. Read-Out	5
4. Contact Us	6
5. Product Use & Liability	6



Please read the entire manual before starting your experiments!
Carefully note the handling and storage conditions.
For research use only.

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}

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) Burkhardt, 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.

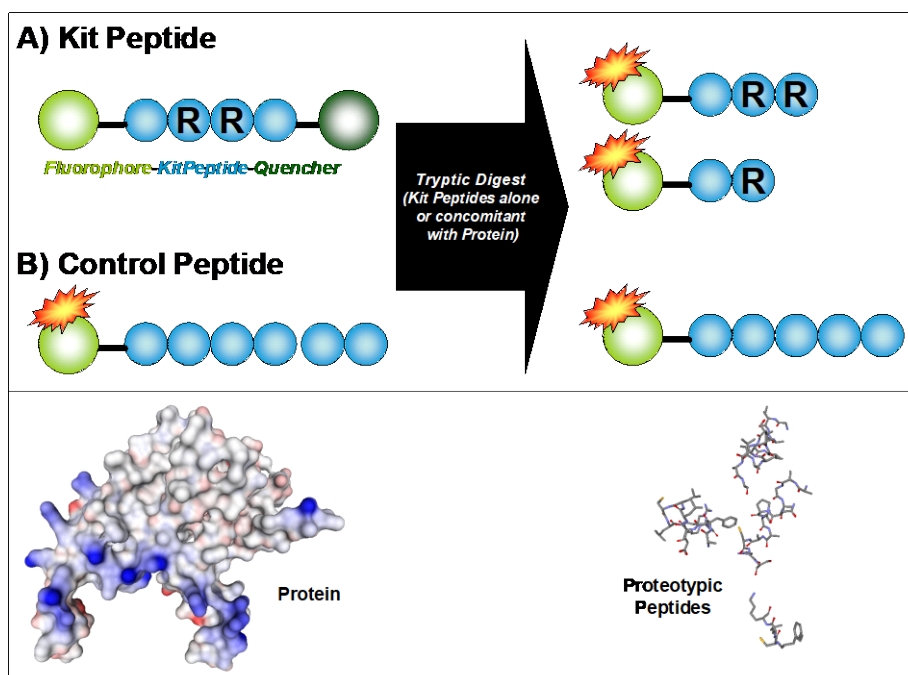
2. TrypCheck Kit_Fluorescence

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.

2.1 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 amount that corresponds 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.

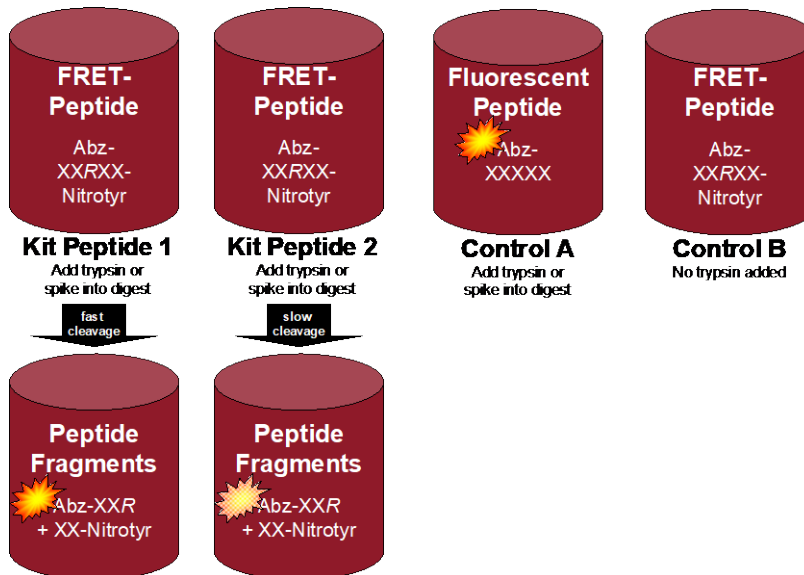


Scheme 1: Tagging & cleavage process (exemplarily for one kit peptide).

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.



2.2 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.

Summary

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

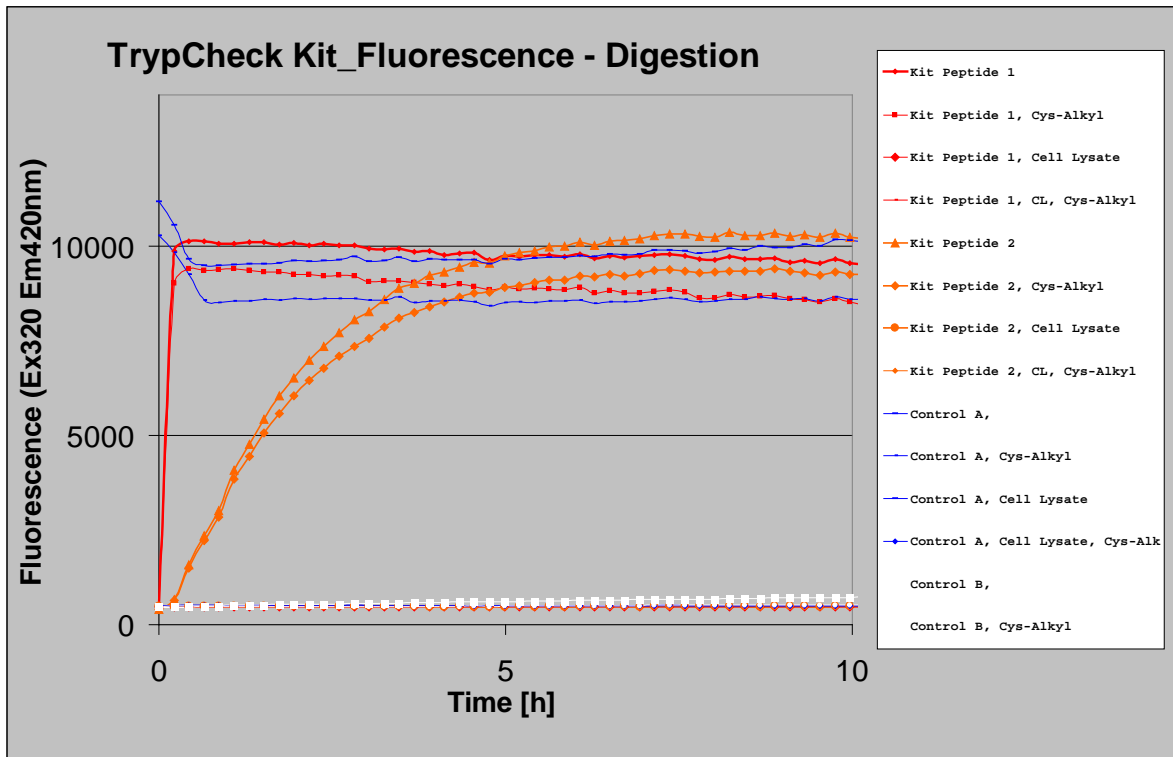


Figure 2: Cleavage of the kit peptides by trypsin followed by fluorescence readout (15 min intervals, 10 hours).

2.3 List of Components

Polypropylene vials with the following compounds:

Name	Sequence	Amount [nmol]	Amount [µg]	Ease of trypsin cleavage
Kit Peptide 1	<u>Abz-ARRA-Nitrotyr-NH₂</u>	200	160	++
Kit Peptide 2	<u>Abz-AVKD-Nitrotyr-NH₂</u>	200	152	+
Control A	<u>Abz-AR-OH</u>	200	73	n/a
Control B	<u>Abz-ARRA-Nitrotyr-NH₂</u>	200	160	n/a

2.4 Storage

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

3. Experimental Protocols



The following procedures are provided only as guidelines. The optimal experimental conditions will vary depending on the sample used.

3.1 Tryptic Digestion

- 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.
- 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
Kit Peptide 1	<u>Abz</u> -ARRA- <u>Nitrotyr</u> -NH ₂	yes
Kit Peptide 2	<u>Abz</u> -AVKD- <u>Nitrotyr</u> -NH ₂	yes
Control A	<u>Abz</u> -AR-OH	yes
Control B	<u>Abz</u> -ARRA- <u>Nitrotyr</u> -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 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 reduction and alkylation steps.

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

3.2 Read-Out

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)	$(X2-CB) / (CA-CB) * 100$

4. Contact Us

Technical Support	Address
Customer Support +49-30-322980-7878 peptide@jpt.com	JPT Peptide Technologies GmbH Hermann-Dorner-Allee 23 12489 Berlin Germany
Technical Support Europe +49-30-322980-7830 peptide@jpt.com	Visit our Website!
Technical Support North America 1.888.JPT.COM0 (1.888.578.2660) us-bd@jpt.com	

5. Product Use & Liability

THE PRODUCTS ARE FOR EXPERIMENTAL LABORATORY USE ONLY AND NOT INTENDED FOR HUMAN OR HOUSEHOLD USE.

Only qualified personnel should handle these chemicals.

Note that missing hazard warnings do not indicate that a product is harmless. Products are for research use only (RUO). JPT Peptide Technologies declines responsibility for any damage arising from the inappropriate use of its products.

JPT Peptide Technologies makes no warranties of any kind, expressed or implied extending beyond the description of the product in this brochure, except that the material will meet our described specifications at the time of delivery.

JPT Peptide Technologies makes no warranties regarding experimental results and assumes no liability for injuries, damages, or penalties resulting from product use since the conditions of handling and use are beyond our control.