

Trypsin Sequencing Grade, modified

From bovine pancreas

Cat. No. 11 418 025 001

4 × 25 µg

Cat. No. 11 418 033 001

4 × 100 µg

 Version 16

Content version: May 2013

Store at +2 to +8°C

1. What this Product Does

Content

Lyophilizate, salt-free.

Source

Trypsin Sequencing Grade, modified is isolated from bovine pancreas as a highly purified and specific protease and subsequently modified.

Storage and Stability

Stable at +2 to +8°C until the expiration date printed on the label. The working solution of Trypsin Sequencing Grade, modified in 1% acetic acid or 1 mM HCl may be used for maximum of one week, when stored at +2 to +8°C.

 Store dry!

Trypsin Sequencing Grade, modified, is more resistant towards autolysis even at pH values in the neutral and weakly basic range (Tab. 1). Therefore, the enzyme can be used in high concentrations in the digestion assay.

Incubation time (h)	Activity	
	Trypsin, modified sequencing grade	Trypsin, native sequencing grade
0	100	100
2	100	48
5	84	17
20	34	2

Tab. 1: Stability of Trypsin Sequencing Grade, modified, and of native trypsin in 1 mM Tris-HCl, pH 8.5 at 37°C.

2. How to Use this Product

2.1 Before You Begin

General Handling Recommendations

The content of one vial may be used for several simultaneous digests. A new vial should be taken when repeating a digest in order to minimize the risk of contamination or autolysis.

Resistance of the enzyme against autolysis in solution is remarkably increased due to modification (cross linking to a hydrophilic polymer).

2.2 Digestion of Proteins in Solution

Working Solution

Reconstitute lyophilized Trypsin Sequencing Grade, modified in 1% acetic acid or 1 mM HCl.

Procedure

- ① Dissolve the proteins to be sequenced in digestion buffer (100 mM Tris-HCl, pH 8.5).
- ② In the case of proteins that are hard to solubilize, add urea, SDS, or guanidine HCl to the digestion buffer prior to solubilizing the protein. When applying urea, Roche recommends also adding 20 mM methylamine.
- ③ To achieve a suitable concentration of the denaturing agent in the digest, the protein solution has to be correspondingly diluted with buffer (Tab. 2).
- ④ The recommended amount of enzyme is 1/100 to 1/5 of the protein by weight.
- ⑤ The incubation time should be chosen between 2 and 18 h at +37°C depending on the amount of enzyme.

Denaturing agent	Concentration	Enzyme activity in %
without addition (control)	-	100
sodium dodecyl sulfate (SDS)	0.001% (w/v)	139
	0.01% (w/v)	153
	0.1% (w/v)	180
urea	0.1 M	170
	0.5 M	150
	1.0 M	100
guanidine hydrochloride	0.1 M	114
	0.5 M	31
	1.0 M	0
acetonitrile	1% (v/v)	260
	5% (v/v)	270
	10% (v/v)	310

Tab. 2: Activity determination of Trypsin Sequencing Grade, modified, with Chromozym TRY in the presence of stated concentrations of denaturing agents.

Incubation of Trypsin Sequencing Grade, modified, 200 µg/ml, with denaturing agent for 6 h at +25°C in 100 mM Tris-HCl, pH 8.5.

 Roche recommends also adding 20 mM methylamine when applying urea.

3. Additional Information on this Product

3.1 Quality Control

Function and purity control by HPLC of each lot ensure a constant quality.

According to the current quality control procedures, the enzyme is free of impurities that might interfere with the separation range of peptides in reversed-phase HPLC (highly sensitive detection at 206 - 230 nm).

Specificity and Nonspecificity Verification

Trypsin Sequencing Grade, modified, is a serine protease that specifically hydrolyzes proteins and peptide bonds C-terminally of lysine and

arginine at pH 7.5-9.0. Amide and ester bonds of Arg and Lys are also cleaved.

The specificity and nonspecificity of Trypsin Sequencing Grade, modified, is verified with the oxidized B-chain of insulin (insulin B_{ox}) as substrate.

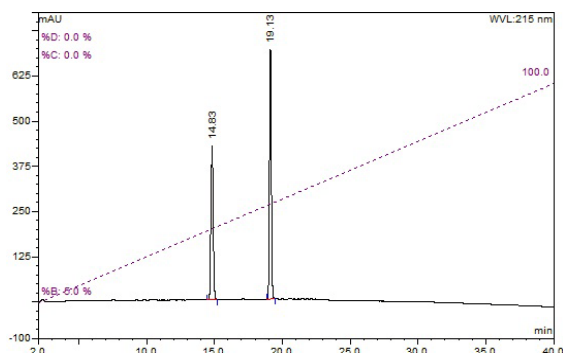


Fig. 1: Specificity of Trypsin Sequencing Grade, modified, in reversed phase HPLC.

High concentrations of Trypsin Sequencing Grade, modified, (1 part by weight enzyme with 18 parts by weight insulin B_{ox}) are incubated for 1 h to detect the fragments of the specific digested substrate.

Digest	180 µg insulin B _{ox} + 10 µg Trypsin Sequencing Grade in 190 µl 100 mM Tris-HCl, pH 8.5, 1 h at +37°C; reversed phase HPLC 10 µl digest diluted with Tris buffer to 40 µl.
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength:	215 nm
Fragments	14.83 min Gly (23) – Lys (29) 19.13 min Phe (1) – Arg (22)

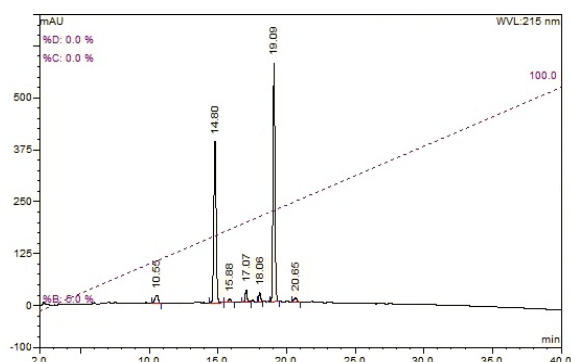


Fig. 2: Nonspecificity of Trypsin Sequencing Grade, modified, in reversed phase HPLC.

High concentrations of Trypsin Sequencing Grade, modified, (1 part by weight enzyme with 18 parts by weight insulin B_{ox}) are incubated for 18 h to detect traces of impurities.

Digest	180 µg insulin B _{ox} + 10 µg Trypsin Sequencing Grade in 190 µl 100 mM Tris-HCl, pH 8.5, 18 h at +37°C; reversed phase HPLC 10 µl digest diluted with Tris buffer to 40 µl.
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength:	215 nm
Fragments	14.80 min Gly (23) – Lys (29) 19.09 min Phe (1) – Arg (22)

4. Supplementary Information

Changes to Previous Version

- Update of Quality control data
- Editorial Changes

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.

Symbols

Symbols are used in this document to highlight important information:

Symbol	Description
📌	Information Note: Additional information about the current topic or procedure.

Ordering Information

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Product	Pack Size	Cat. No.
Denaturation Reagents		
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Urea EP-MB grade	1 kg	11 685 899 001
Sodium Dodecyl Sulfate	1 kg	11 667 289 001

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