

Trypsin sequencing grade

From bovine pancreas

Cat. No. 11 418 475 001

4 × 25 µg

Cat. No. 11 047 841 001

4 × 100 µg

Version November 2006

Store at +2 to +8°C

1. What this Product Does

Content

Lyophilizate, salt-free.

Storage and Stability

Stable at +2 to +8°C until the expiration date printed on the label.

☞ Store dry!

A solution in 0.01% trifluoroacetic acid (TFA), (v/v) or 1 mM HCl may be used for one week at maximum, if stored at +2 to +8°C. By incubation of proteins in solution at neutral to slightly basic pH-values partial autolysis might occur. For this application we recommend trypsin, modified, sequencing grade*.

Application

Trypsin sequencing grade is suitable to digest proteins in solution, in gels or on blotting membranes.

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. In order to repeat the digest a new vial should be taken. Thereby the utmost reproducibility can be guaranteed and probable contamination or autolysis will be avoided.

2.2 Digestion of Proteins in Solution

Working Solution

Lyophilized Trypsin sequencing grade is reconstituted in 0.01% trifluoroacetic acid (TFA) (v/v), or 1 mM HCl.

Handling of Proteins

- ① The proteins, to be sequenced, are dissolved in digestion buffer (100 mM Tris-HCl, pH 8.5).
- ② In the case of proteins which are hard to solubilize, urea, SDS or guanidine HCl should be added to the digestion buffer prior to solubilization of the protein. On application of urea it is recommended to add also 20 mM methylamine.
- ③ In order to achieve a suitable concentration of the denaturing agent in the digest, the protein solution has to be correspondingly diluted with buffer (table)
- ④ The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.

Tab. 1: Activity determination of Trypsin sequencing grade with Chromozym TRY in the presence of stated concentrations of denaturing agents. Incubation of Trypsin sequencing grade 200 µg/ml, with denaturing agent for 6 h at +25°C in 100 mM Tris-HCl, pH 8.5.

☞ On application of urea it is recommended to also add 20 mM methylamine.

Denaturing agent	Concentration	Enzyme activity in %
without addition (control)	-	100
sodium dodecyl sulfate (SDS)*	0.001% (w/v)	120
	0.01% (w/v)	110
	0.1% (w/v)	105
urea*	0.1 M	86
	0.5 M	86
	1.0 M	90
guanidine hydrochloride*	0.05 M	62
	0.1 M	33
	0.3 M	6
	0.5 M	4
acetonitrile	1% (v/v)	100
	5% (v/v)	114
	10% (v/v)	134

2.3 Digestion of Proteins in Gels or on Blotting Membranes

Working Solution

Trypsin sequencing grade is first dissolved with 1 mM HCl to a concentration of 0.1 mg/ml and further diluted with digestion buffer (50 mM ammonium hydrogencarbonate or 100 mM Tris-HCl, pH 8.5) to 1 - 5 µg Trypsin in 100 µl immediately before use.

☞ In order to stabilize the trypsin calcium chlorid (1 mM) can be added to the digestion buffer.

Procedure

Several protocols describing the cleavage of proteins in gels or on membranes have been published (1-5).

As much volume is added to the gel as every shrinked piece becomes completely reswollen and covered. For the incubation of proteins on blotting membranes detergents like Triton X-100 or PVP-40 are added to the digestion buffer which should just completely cover the membrane piece.

A parallel control incubation using a gel or membrane piece of about the same size but without protein is recommended for each experiment. This facilitates the detection of artefacts due to the gel, membrane or staining as well as to a possible autolysis of the trypsin.

Incubation Time

The incubation time should be chosen between 2 and 18 h at 37°C depending on the amount of protein to be digested.

3. Additional Information on this Product

3.1 Product Characteristics

Source

Trypsin sequencing grade is isolated from bovine pancreas as a highly purified and specific protease.

Molecular Weight

23,500 Da

Sequence of β -trypsin

[Titani, K. et al. (1975) *Biochemistry* 14, 1358-1366].

IVGG	YTCGANTVPY	QVSLNSGYHF	CGGSLINSQW	VVSAAHCKYS
GIQVRLGEDN	INVVEGNEQF	ISASKSIVHP	SYNSNTLNND	IMLIKLSAA
SLNSRVASIS	LPTSCASAGT	QCLISGWGNT	KSSGTSYPDV	LKCLKAPILS
DSSCKSAYPG	QITSNMFCAG	YLEGGKDSCQ	GDSGGFVVC	GKLQGIWSWG
SGCAQKNKPG	VYTKVCNYVS	WIKQTIASN		

Specificity

Trypsin sequencing grade is a serine protease.

It specifically cleaves peptide bonds C-terminally at lysine and arginine. The specificity of Trypsin sequencing grade is verified with the oxidized B-chain of insulin (insulin B_{ox}) as substrate. High concentrations of Trypsin sequencing grade (1 part by weight enzyme with 18 parts by weight insulin B_{ox}) are incubated for 18 h to detect traces of impurities of chymotrypsin (Fig 1).

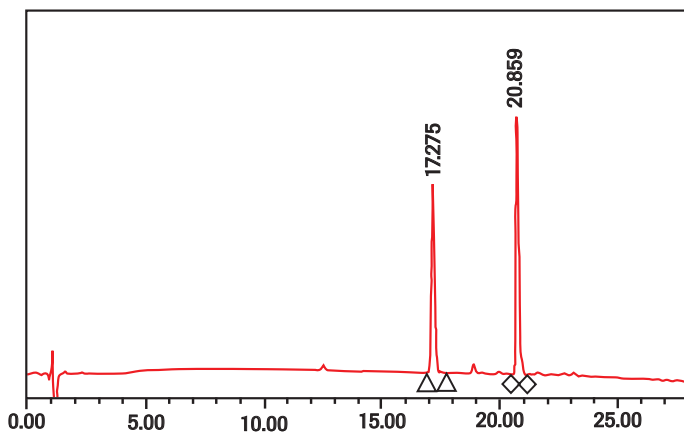


Fig. 1: Specificity of Trypsin sequencing grade in reversed phase HPLC.

Digest	100 μ g insulin B _{ox} + 5.6 μ g Trypsin sequencing grade in 100 μ l 100 mM Tris-HCl, pH 8.5. 18 h at +37°C; reversed phase HPLC 10 μ l digest diluted with Tris buffer to 100 μ l.
Column	Polygosil C18
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 50% acetonitrile (v/v)
Gradient	30 min linearly 0-100% B;
Flow rate	1 ml/min
Wave length:	215 nm
Fragments	21.7 min Gly (23) – Lys (29) 24.8 min Phe (1) – Arg (22)

References

- 1 Kellner, R. (1995) *Biochemica* No. 2, Roche Applied Science.
- 2 Jenö, et al. (1995) *Anal. Biochem.* **224**, 75-82.
- 3 Shevchenko, A. et al. (1996) *Anal. Chem.* **68**, 850-858.
- 4 Eckerskorn, F. & Lottspeich, F. (1991) in: *Electrophoresis Forum* (Radola, B-J-, Hrsg.) S. 283-288.
- 5 Fernandez, J. et al. (1994) *Anal. Biochem.* **21**, 112-117.

3.2 Quality Control

The enzyme is free of impurities, which might interfere in the separation range of peptides in reversed phase HPLC (highly sensitive detection at 206 – 230 nm).

Function and purity control by HPLC ensure a constant quality (Fig 2).

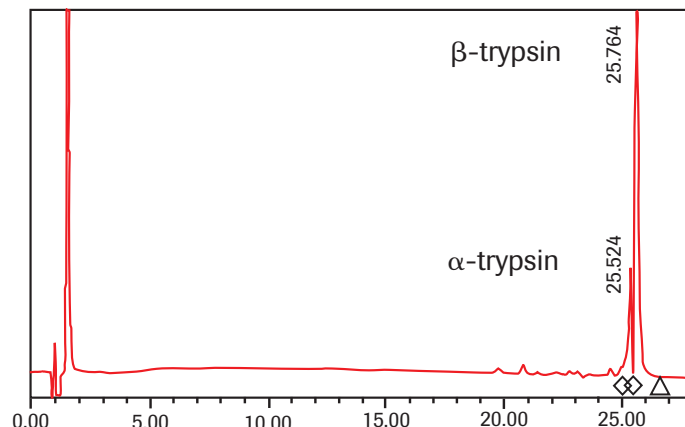


Fig. 2: Purity of Trypsin sequencing grade in reversed phase HPLC.

Quantity	20 μ g Trypsin sequencing grade
Volume	20 μ l
Column	Aquapore RP 300 4.6 $\times$ 30 mm, 7 μ
Solvent A	0.1% trifluoroacetic acid (TFA) (v/v) in water
Solvent B	0.1% TFA, (v/v) in water; 70% acetonitrile (v/v)
Gradient	30 min linear 0 – 100%
Flow rate	0.5 ml/min;
Wave length:	215 nm

4. Supplementary Information

Changes to Previous Version

- Revised sequence of β -trypsin
- new layout.
- minor editorial changes.

Text Conventions

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

Text Convention	Use
Numbered instructions labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

Symbols are used in this Instruction Manual to highlight important information:

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

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com

Product	Pack Size	Cat. No.
Proteases Sequencing Grade		
Carboxypeptidase Y Sequencing Grade	3 × 20 μ g	11 111 914 001
Chymotrypsin Sequencing Grade	4 × 25 μ g	11 418 467 001
Endoproteinase Arg-C Sequencing Grade	3 × 5 μ g	11 370 529 001
Endoproteinase Asp-N Sequencing Grade	2 μ g 3 × 2 μ g	11 420 488 001 11 054 589 001
Endoproteinase Glu-C Sequencing Grade	50 μ g 3 × 50 μ g	11 420 399 001 11 047 817 001
Endoproteinase Lys-C Sequencing Grade	5 μ g 3 × 5 μ g	11 420 429 001 11 047 825 001
Trypsin, modified Sequencing Grade	4 × 25 μ g 4 × 100 μ g	11 418 025 001 11 418 033 001
Denaturation Reagents		
Guanidine hydrochloride	500 g	11 492 942 001
Guanidin thiocyanate crystallized	500 g	11 685 929 001
Urea EP-MB grade	1 kg	11 685 899 001
Sodium Dodecyl Sulfate	1 kg	11 667 289 001

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Triton is a Trademark of Rohm & Haas Company, Philadelphia, PA, USA.

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