Print

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 23
Content version: April 2013
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!

The working solution of Trypsin Sequencing Grade in 0.01% trifluoroacetic acid (TFA), (v/v) or 1 mM HCl may be used for maximum of one week, when stored at +2 to +8°C. Partial autolysis may occur when incubating proteins in solution at neutral to slightly basic pH-values. For this application, we recommend Trypsin Sequencing Grade.

Application

Trypsin Sequencing Grade is suitable for digesting proteins in solution, 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. A new vial should be taken when repeating a digest in order to minimize the risk of contamination or autolysis.

2.2 Digestion of Proteins in Solution

Working Solution

Reconstitute lyophilized Trypsin Sequencing Grade in 0.01% trifluoroacetic acid (TFA) (v/v), 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 (Table 1).

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.

Roche recommends also adding 20 mM methylamine when applying urea.

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 chloride (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 such as Triton X-100 or PVP-40 are added to the digestion buffer to 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].

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      IVGG   YTCGANTVPY   QVSLNSGYHF   CGGSLINSQW   VVSAAHCYKS
GIQVRLGEDN   INVVEGNEQF   ISASKSIVHP   SYNSNTLNND   IMLIKLKSAA
SLNSRVASIS   LPTSCASAGT   QCLISGWGNT   KSSGTSYPDV   LKCLKAPILS
DSSCKSAYPG   QITSNMFCAG   YLEGGKDSCQ   GDSGGPVVCS   GKLQGIVSWG
SGCAQKNKPG   VYTKVCNYVS   WIKQTIASN
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3.2 Quality Control

Performance and purity are checked with HPLC.

Specificity and Nonspecificity Verification

Trypsin Sequencing Grade is a serine protease that specifically cleaves peptide bonds C-terminally at lysine and arginine. The specificity and unspecificity of Trypsin Sequencing Grade is verified using the oxidized B-chain of insulin (insulin B_{ox}) as the substrate.

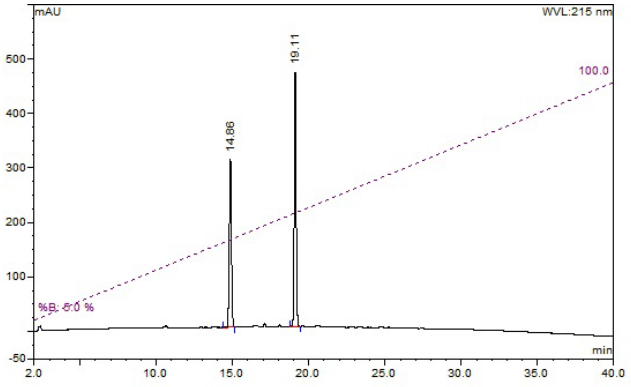

Fig. 1: Specificity of Trypsin Sequencing Grade in reversed phase HPLC. High concentrations of Trypsin Sequencing Grade (1 part by weight enzyme with 18 parts by weight insulin B_{ox}) are incubated for 1 h to detect the fragments of the specific digested substrate.

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 ml 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	14.86 min Gly (23) – Lys (29) 19.11 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: *Electrophorese Forum* (Radola, B-J-, Hrsg.) S. 283-288.
5 Fernandez, J. et al. (1994) *Anal. Biochem.* **21**, 112-117.

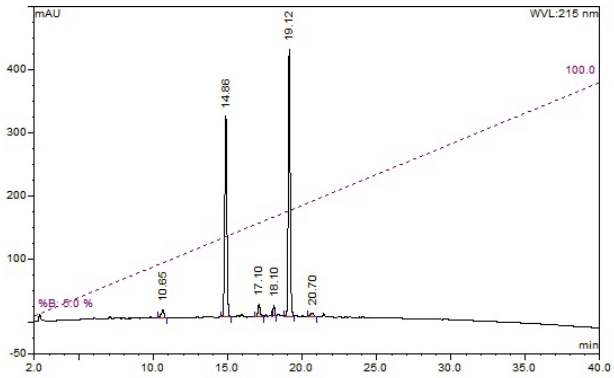

Fig. 2: Nonspecificity of Trypsin Sequencing Grade in reversed phase HPLC. High concentrations of Trypsin Sequencing Grade (1 part by weight enzyme with 18 parts by weight insulin B_{ox}) are incubated for 1 h to detect traces of impurities of Trypsin.

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	14.86 min Gly (23) – Lys (29) 19.12 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.

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Sodium Dodecyl Sulfate	1 kg	11 667 289 001

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