For life science research only.
Not for use in diagnostic procedures.

Trypsin Sequencing Grade
from bovine pancreas

Version: 25
Content Version: November 2020

Lyophilized

Cat. No. 11 418 475 001	4 x 25 µg
Cat. No. 11 047 841 001	4 x 100 µg

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Trypsin Sequencing Grade	▪ Highly purified and specific protease. ▪ Salt-free	11 418 475 001	4 vials, 25 µg each
			11 047 841 001	4 vials, 100 µg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Trypsin Sequencing Grade	Store at +2 to +8°C. ⚠ ***Store dry.***

1.3. Additional Equipment and Reagent required

For preparation of lyophilizate

- 0.01% trifluoroacetic acid (TFA) (v/v), or
- 1 mM HCl

For digestion of proteins in solution

- Tris-HCl*
- Sodium dodecyl sulfate (SDS*)
- Urea
- Guanidine hydrochloride
- Acetonitrile

For digestion of proteins in gels or on blotting membranes

- Tris-HCl*
- Ammonium hydrogen carbonate
- Calcium chloride
- Triton X-100* or PVP-40

1.4. Application

Use Trypsin Sequencing Grade for digesting proteins
- in solution,
- gels,
- or on blotting membranes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

General handling

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

Activity determination

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 hours at +25°C in 100 mM Tris-HCl, pH 8.5.
i *Add 20 mM methylamine when applying urea.*

Denaturing agent	Concentration	Enzyme activity in [%]
without addition (control)	–	100
SDS	0.001% (w/v)	120
	0.01% (w/v)	110
	0.1% (w/v)	105
Urea (+ methylamine)	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

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation/Composition	Storage and Stability	For use in...
Trypsin Sequencing Grade	Dissolve lyophilizate in 0.01% trifluoroacetic acid (TFA) (v/v), or 1 mM HCl. *Partial autolysis may occur when incubating proteins in solution at neutral to slightly basic pH-values.*	Store 1 week at +2 to +8°C.	Digestion mixture of proteins in solution.
	Dissolve lyophilizate with 1 mM HCl to a concentration of 0.1 mg/ml. *To stabilize the trypsin, 1 mM calcium chloride can be added to the digestion buffer.*		Digestion mixture of proteins in gels or on blotting membranes.
Digestion buffer	100 mM Tris-HCl, pH 8.5	–	Dissolution of the proteins in solution.
	50 mM ammonium hydrogen carbonate or 100 mM Tris-HCl, pH 8.5.	–	Dissolution of the proteins in gels or on blotting membranes.

2.2. Protocols

Digestion of proteins in solution

See section, **Working Solution** *for information on preparing solutions.*

1. Dissolve the proteins to be sequenced in Digestion buffer.
   For proteins that are hard to solubilize, add urea, SDS, or guanidine hydrochloride to the Digestion buffer prior to solubilizing the protein. When applying urea, also add 20 mM methylamine.

2. Dilute protein solution with buffer, see section, **General Considerations** to achieve a suitable concentration of the denaturing agent in the digest.
   The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.

3. Choose an incubation time between 2 and 18 hours at +25°C, depending on the amount of enzyme.

Digestion of proteins in gels or on blotting membranes

Several protocols describing the cleavage of proteins in gels or on membranes have been published.

1. Perform electrophoresis and use the gel or transfer the proteins to an appropriate membrane.
   – Incubate the gel or the membrane as shown in the table:

Incubation of proteins	Procedure
in gels	Add as much volume of Digestion buffer to the gel as every shrinked piece becomes completely reswollen and covered.
on membranes	Add detergents such as Triton X-100* or PVP-40 to the Digestion buffer and completely cover the membrane piece.

2. Add in parallel, a control incubation.
   – Use a gel or membrane piece of about the same size but without protein for each experiment.
   This facilitates the detection of artifacts due to the gel, membrane, or staining, as well as to a possible autolysis of the trypsin.

3. Chose an incubation time between 2 and 18 hours at +37°C, depending on the amount of protein to be digested.

2.3. Parameters

Molecular Weight

23,500 Da

Sequence

Sequence of β-trypsin

1				
IVGGYTCGAN	TVPYQVSLNS	GYHFCGGSLI	NSQWVVSAAH	CYKSGIQVRL
51				
GEDNINVVEG	NEQFISASKS	IVHPSYNSNT	LNNDIMLIKL	KSAASLNSRV
101				
ASISLPTSCA	SAGTQCLISG	WGNTKSSGTS	YPDVLKCLKA	PILSDSSCKS
151				
AYPGQITSNM	FCAGYLEGGK	DSCQGDSGGP	VVCSGKLQGI	VSWGSGCAQK
201				
NKPGVYTKVC	NYVSWIKQTI	ASN		

3. Additional Information on this Product

3.1. Test Principle

Background information

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

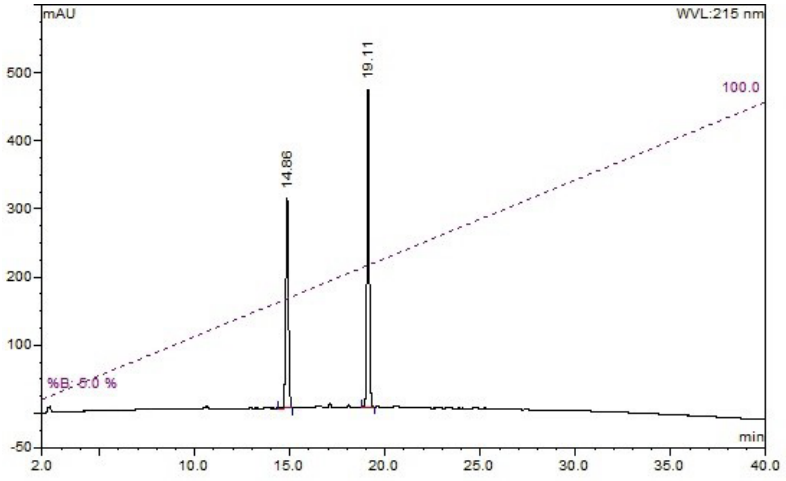

Fig. 1: Specificity of Trypsin Sequencing Grade in reversed phase HPLC.
High concentrations of Trypsin Sequencing Grade (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated for 1 hour to detect the fragments of the specific digested substrate.

Digest	180 µg insulin B_{ox} + 10 µg Trypsin Sequencing Grade in 190 µl 100 mM Tris-HCl, pH 8.5; 1 hour at +37°C; reversed phase HPLC: 10 µl digest diluted with Tris buffer to 40 µl.
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
Wavelength	215 nm
Fragments	14.86 minute Gly (23) – Lys (29) 19.11 minute Phe (1) – Arg (22)

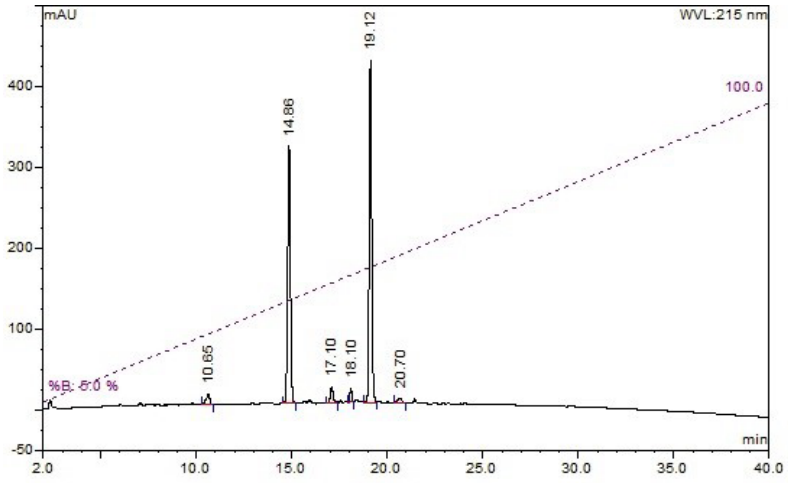

Fig. 2: Nonspecificity of Trypsin Sequencing Grade in reversed phase HPLC.
High concentrations of Trypsin Sequencing Grade (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated for 18 hours to detect traces of impurities.

Digest	180 µg insulin B_{ox} + 10 µg Trypsin Sequencing Grade in 190 µl 100 mM Tris-HCl, pH 8.5; 18 hours at +37°C; reversed phase HPLC: 10 µl digest diluted with Tris buffer to 40 µl.
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
Wavelength	215 nm
Fragments	14.86 minute Gly (23) – Lys (29) 19.12 minute Phe (1) – Arg (22)

3.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tris hydrochloride	500 g	10 812 846 001
Triton X-100	50 ml, 5 x 10 ml	11 332 481 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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