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

Endoproteinase Glu-C Sequencing Grade

from *Staphylococcus aureus* V8

Version: 21

Content Version: November 2020

Lyophilized

Cat. No. 11 420 399 001	50 µg
Cat. No. 11 047 817 001	3 x 50 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	Endoproteinase Glu-C Sequencing Grade	▪ Highly purified and specific protease. ▪ Salt-free	11 420 399 001	1 vial, 50 µg
			11 047 817 001	3 vials, 50 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	Endoproteinase Glu-C Sequencing Grade	Store at +2 to +8°C. ⚠ ***Store dry.***

1.3. Additional Equipment and Reagent required

For preparation of digestion buffer

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

- Ammonium carbonate buffer

For solubilization of proteins

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

1.4. Application

Use Endoproteinase Glu-C for the specific cleavage of proteins and peptides for:

- Protein structure
- Sequence analysis

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 Endoproteinase Glu-C, with Z-Phe-Leu-Glu-4-nitranilide as substrate in the presence of stated concentrations of denaturing agents. Incubation of Endoproteinase Glu-C, 200 µg/ml, with denaturing agent for 6 hours at +25°C in 25 mM sodium phosphate, pH 7.8.

i Add 20 mM methylamine when applying urea.

Denaturing agent	Concentration	Enzyme activity [%]
without addition (control)	–	100
SDS	0.001% (w/v)	106
	0.01% (w/v)	110
	0.1% (w/v)	77
Urea (+ methylamine)	0.1 M	102
	0.5 M	90
	1.0 M	82
Guanidine hydrochloride	0.1 M	94
	0.5 M	98
	1.0 M	94
Acetonitrile	1% (v/v)	110
	5% (v/v)	151
	10% (v/v)	118

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...
Endoproteinase Glu-C Sequencing Grade	▪ Add double-distilled water to the lyophilizate. ⚠ ***In order to avoid autolysis, the incubation temperature should not exceed +25°C.***	Store 2 days at +2 to +8°C.	Digestion mixture
Digestion buffer	25 mM ammonium carbonate buffer, pH 7.8.	–	Dissolution of the proteins to be sequenced.

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.

If Endoproteinase Glu-C is incubated in the presence of organic cosolvents such as n-propanol, the enzyme shows synthetic activity. Two fragments of hemoglobin S α-chain, α_{1-30} and α_{31-47} are covalently complexed by incubation with Endoproteinase Glu-C at pH 6.0 for 24 hours at +4°C in the presence of 25% n-propanol to form α_{1-47}. The yield is about 50%.

2.3. Parameters

Molecular Weight

30 kDa

Sequence

Sequence of Endoproteinase Glu-C

1 MKGKFLKVSS LFVATLTTAT LVSSPAANAL SSKAMDNHPQ QTQSSKQQTP
51 KIQKGGNLKP LEQREHANVI LPNNDRHQIT DTTNGHYAPV TYIQVEAPTG
101 TFIASGVVVG KDTLLTNKHV VDATHGDPHA LKAFPSAINQ DNYPNGGFTA
151 EQITKYSGEG DLAIVKFSPN EQNKHIGEVV KPATMSNNAE TQVNQNITVT
201 GYPGDKPVAT MWESKGKITY LKGEAMQYDL STTGGNSGSP VFNEKNEVIG
251 IHWGGVPNEF NGAVFINENV RNFLKQNIED IHFANDDQPN NPDNPDNPNN
301 PDNPNNPDEP NNPDNPNNPD NPDNGDNNNS DNPDAA

3. Additional Information on this Product

3.1. Test Principle

Background information

Endoproteinase Glu-C is a serine protease that specifically cleaves peptide bonds C-terminally at glutamic acid and with a 3,000-fold lower rate at aspartic acid. The apparent specificity for glutamic acid is higher in ammonium carbonate buffer, pH 7.8 and ammonium acetate buffer, pH 4.0. The specificity and nonspecificity of Endoproteinase Glu-C is verified with the oxidized B-chain of insulin (insulin B_{ox}) as substrate.

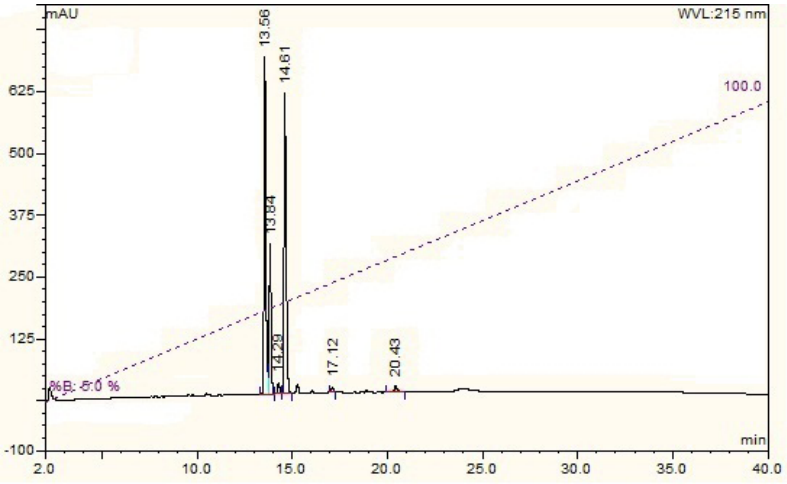

Fig. 1: Specificity of Endoproteinase Glu-C in reversed phase HPLC.
High concentrations of Endoproteinase Glu-C (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated for 1 hour to detect the fragments of the specific digested substrate.

Digest	100 µg insulin B_{ox} + 10 µg Endoproteinase Glu-C in 100 µl 25 mM ammonium carbonate buffer, pH 7.8; 1 hour at +25°C; reversed phase HPLC: 10 µl digest diluted with ammonium carbonate buffer to 110 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	13.56 minutes Phe (1) – Glu (13) 13.84 minutes Ala (14) – Glu (21) 14.61 minutes Arg (22) – Ala (30)

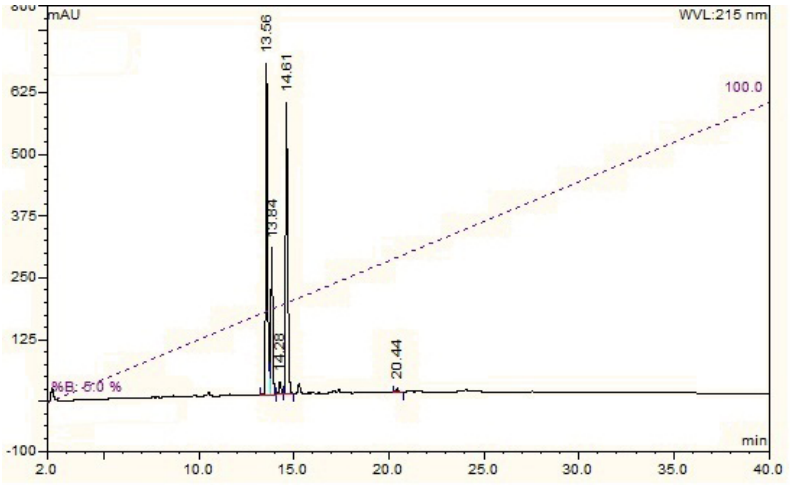

Fig. 2: Nonspecificity of Endoproteinase Glu-C in reversed phase HPLC.
High concentrations of Endoproteinase Glu-C (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated for 18 hours to detect traces of impurities.

Digest	100 µg insulin B_{ox} + 10 µg Endoproteinase Glu-C in 100 µl 25 mM ammonium carbonate buffer, pH 7.8; 18 hours at +25°C; reversed phase HPLC: 10 µl digest diluted with ammonium carbonate buffer to 110 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	13.56 minutes Phe (1) – Glu (13) 13.84 minutes Ala (14) – Glu (21) 14.61 minutes Arg (22) – Ala (30)

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

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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