

Endoproteinase Glu-C

(Protease V 8) sequencing grade
From *Staphylococcus aureus* V 8

Cat. No. 11 420 399 001

50 µg

Cat. No. 11 047 817 001

3 × 50 µg

Version March 2010

Store at 2-8° C

Commercial availability

Lyophilisate, salt-free.

Endoproteinase Glu-C sequencing grade is isolated from *Staphylococcus aureus* V 8 as a highly purified and specific protease ($M_r = 30$ kD).

Purity

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. 1).

Specificity

Endoproteinase Glu-C sequencing grade is a serine protease, cleaving peptide bonds C-terminally at glutamic acid and with a 3000-fold lower rate at aspartic acid (1). The apparent specificity for glutamic acid is higher in ammonium carbonate buffer, pH 7.8 and ammonium acetate buffer, pH 4.0 (2, 3).

The specificity of endoproteinase Glu-C sequencing grade is verified with the oxidized B-chain of insulin (insulin B_{ox}) as substrate. High concentrations of endoproteinase Glu-C sequencing grade (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated to detect traces of impurities e.g. contaminating proteases (fig. 2).

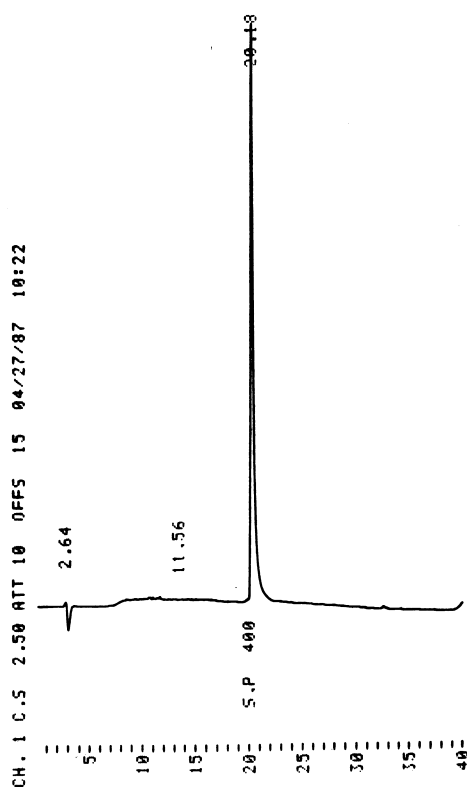


Figure 1: Purity of endoproteinase Glu-C sequencing grade in reversed phase HPLC.

Quantity: 20 µg endoproteinase Glu-C sequencing grade;
Volume: 20 µl; column: Aquapore RP 300 4.6 × 100 mm, 7 µm;
solvent A: trifluoroacetic acid (TFA), 0.1% (v/v);
gradient: 30 min linear 0–100% B; flow rate: 0.5 ml/min;
wave length: 215 nm.

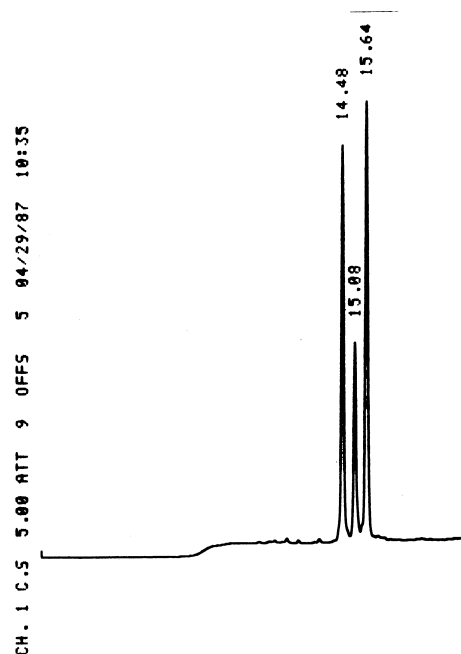


Figure 2: Specificity of endoproteinase Glu-C sequencing grade in reversed phase HPLC.

Digest: 100 µg insulin B_{ox} + 10 µg endoproteinase Glu-C sequencing grade in 100 µl 25 mM ammonium carbonate buffer, pH 7.8; 18 h at 25° C; chromatography: 10 µl digest diluted with ammonium carbonate buffer to 100 µl; column: Aquapore RP 300, 4.6 × 100 mm, 7 µm; solvent A: TFA, 0.1% (v/v) in water; solvent B: TFA, 0.1% (v/v) in water; acetonitrile, 70% (v/v); gradient: 0–15 min 40% B, 15–20 min 100% B, 20–25 min 100% B, 25–27 min 0% B, flow rate: 0.5 ml/min; wave length: 215 nm.

Fragments: 14.48 min Phe(1) – Glu(13),
15.08 min Ala(14) – Glu(21),
15.64 min Arg(22) – Ala(30).

**Storage/
Stability**

The lyophilisate is stable at 2-8°C until the expiration date printed on the label. Once opened the lyophilisate should be stored dry. A solution in double dist. Water may be used for 1-2 days at maximum, if stored at 2-8°C. **Note:** The content of one vial may be used for several simultaneous digests. In order to repeat the digest a new vial should be taken. Therefore the utmost reproducibility, can be guaranteed and probable contamination or autolysis will be avoided.

Reconstitution

Lyophilized Endoproteinase Glu-C sequencing grade is reconstituted in double dist. water. In order to avoid autolysis the incubation temperature should not exceed 25°C.

**Protein
preparation**

The proteins, to be sequenced, are dissolved in digestion buffer (25 mM ammonium carbonate buffer, pH 7.8). 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 also add 20 mM methylamine. 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).

**Handling
instructions**

- The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.
- The incubation time should be chosen between 2 and 18 hours at 25°C depending on the amount of enzyme.

Note: If Endoproteinase Glu-C is incubated in the presence of organic cosolvents (like n-propanol) the enzyme shows synthetic activity (5). 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 h at 4°C in the presence of 25% n-propanol to form α_{1-47} . The yields is about 50% (5).

**Activity
determination**

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

Denaturing agent	Concentration	Enzyme activity in %
without addition (control)	-	100
sodium dodecyl sulfate (SDS)	0.001% (w/v)	106
	0.01 % (w/v)	110
	0.1 % (w/v)	77
urea	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

Sequence of endoproteinase Glu-C (3):

1 MKGKFLKVSS LFMATLTAT LVSSPAANAL SSKAMDNHPQ QTQSSKQQT
51 KIQKGGNLKP LEQREHANVI LPNDRHQIT DTTNGHYAPV TYIQVEAPTG
101 TFIASGVVVG KDTLLTNKHV VDATHGDPHA LKAFPSAINQ DNYPNGGFTA
151 EQITKYSGEG DLAIKVFSPN EQNKHIGEVV KPATMSNNAE TQVNQNITVT
201 GYPGDKPVAT MWESK GKITY LKGEAMQYDL STTGGNSGSP VFNEKNEVIG
251 IHWGGVPNEF NGAVFINENV RNFLKQNIED IHFANDDQPN NPDNPDNPN
301 PDNPNPDEP NPDNPNPNPD NPDNGDNNS DNPDA

Related products, available from Roche Applied Science

Product	Packsize	Cat. No.
Endoproteinases		
Chymotrypsin sequencing grade	4 × 25 µg	11 418 467 001
Endoproteinase Arg-C sequencing grade	3 × 5 µg	11 370 529 001
Endoproteinase Asp-N sequencing grade	2 µg	11 420 488 001
	3 × 2 µg	11 054 589 001
Endoproteinase Lys-C sequencing grade	5 µg	11 420 429 001
	3 × 5 µg	11 047 825 001
Trypsin sequencing grade	4 × 25 µg	11 418 475 001
	4 × 100 µg	11 047 841 001
Trypsin, modified, sequencing grade	4 × 25 µg	11 418 025 001
	4 × 100 µg	11 418 033 001
Trypsin, recombinant, proteomics grade	3 × 20 µg	03 357 228 001
	10 × 20 µg	03 357 236 001
Peptide Mapping Set, contains 4 sequencing grade Endoproteinases: • Trypsin (25 µg) • Endoproteinase Asp-N (2 µg) • Endoproteinase Glu-C (50 µg) • Endoproteinase Lys-C (5 µg)	1 set	11 520 423 001
Denaturing Agents		
Guanidin hydrochloride, crystalline	500 g	11 492 942 001
Sodium dodecyl sulfate, special quality for protein chemistry	500 g	11 028 693 001
Urea, EP-MB-grade	1 kg	11 685 899 001
	5 kg	11 685 902 001
Protease Inhibitors		
Complete ¹⁾	20 tablets	11 697 498 001
Protease Inhibitor Cocktail Tablets	3 × 20 tablets	11 836 145 001
Complete, EDTA-free	20 tablets	11 873 580 001
Complete, Mini	25 tablets	11 836 153 001
Complete, Mini, EDTA-free	25 tablets	11 836 170 001
Protease Inhibitors Set	1 set	11 206 893 001
APMSF	10 mg	10 917 575 001
Aprotinin	10 mg	10 236 624 001
	50 mg	10 981 532 001
	100 mg	11 583 794 001
Pefabloc ²⁾ SC	100 mg	11 429 868 001
	500 mg	11 585 916 001
	1 g	11 429 876 001
PMSF	1 g	10 236 608 001
	10 g	10 837 091 001
	25 g	11 359 061 001

¹⁾ Complete is a trademark of Roche

²⁾ Pefabloc is a trademark of Pentapharm AG, Basel, Switzerland.

References

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