

Endoproteinase GluC



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P8100S 004130314031

P8100S



50 µg Lot: 0041303

Store at -20°C Exp: 3/14

Description: Endoproteinase GluC (*Staphylococcus aureus* Protease V8) is a serine proteinase which selectively cleaves peptide bonds C-terminal to glutamic acid residues (1). Endoproteinase GluC also cleaves at aspartic acid residues at a rate 100–300 times slower than at glutamic acid residues (2,3).

Source: *Staphylococcus aureus* Protease V8 gene cloned and expressed in *Bacillus subtilis*

Applications:

- Digestion of proteins for proteomic analysis by Mass Spectrometry
- Protein and peptide identification

Reaction Conditions: 1X GluC Reaction Buffer.
Incubate at 37°C.

Reagents Supplied with Enzyme:
2X GluC Reaction Buffer.

1X GluC Reaction Buffer:
50 mM Tris-HCl
0.5 mM Glu-Glu
pH 8.0 @ 25°C

Specific Activity: 38.3 µmol/min/mg

Molecular Weight: 29849 daltons

Reconstitution: Endoproteinase GluC should be reconstituted by the addition of 50–500 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

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Storage Conditions: Supplied freeze-dried from a Tris-HCl and sodium chloride buffer.

Can be stored frozen in solution at -20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

Quality Assurance: Endoproteinase GluC is free of glycerol and detergents which may interfere with Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) Mass Spectrometry (MS) or liquid chromatography (LC) methods.

Quality Controls

Endoproteinase GluC Activity:
Measured in three assays:

1. Protein digestion and analysis by MALDI-TOF MS
2. Peptide digestion and analysis by MALDI-TOF MS
3. Fluorometric substrate digestion and specific activity determination in digestion buffer: 50 mM Tris-HCl (pH 8.0) and 0.5 mM Glu-Glu.

Protein Digestion: *Issatchenkia orientalis*

Cytochrome c (Swiss-Prot: CYC_ISSOR) (Sigma) is subjected to digestion by GluC at a ratio of 20:1 respectively for 16 hours at 37°C in GluC Reaction

(see other side)

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Buffer. 1 µl of the above reaction (50 ng) was mixed with 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution, air-dried and subjected to MALDI-TOF MS analysis on an ABI Voyager DE MALDI-TOF MS.

Peptide Digestion: ACTH (1–17) peptide is subjected to digestion by GluC at a ratio of 20:1 respectively for 16 hours at 37°C in GluC Reaction Buffer. 1 µl of the above reaction (10 ng) was mixed with 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution, air-dried and subjected to MALDI-TOF MS analysis.

Fluorometric Assay: 1 µg (~1 µmol) of Anthranilyl-Ala-Phe-Ala-Phe-Glu-Val-Phe(NO₂)-Tyr-Asp peptide (Sigma) was suspended in 150 µl of GluC Reaction Buffer and 1 µg of Endoproteinase GluC was added (4). The initial rate was determined by measurement of the increase in fluorescence (excitation 330 nm and emission 450 nm). The protein concentration is determined by C18 reverse-phase HPLC and integration.

CERTIFICATE OF ANALYSIS

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Endoproteinase GluC Protein Sequence:

1 VILPNNDRHQITD TTNGHYAPVTYIQVEAPTGT FIASGVVVGKDTLLTNKHVV DATHGDP
61 HALKAFPSAINQD NYPNGGFTAEQITKYS GEGDLAIVK FSPNEQNKHIGEVV KPATMSNN
121 AETQVNQNITVT GYPGDKPVATM WESK GKITYLKGEAM QYDLSTTGGNS GSPVFNEKNEV
181 IGIHWGGVPNEF NGAVFINENVR NFKQNI EDIHFANDD QPNNPDNPDN PNNPDNPNPD
241 EPNNPDNPNPD NPDNGDNNNSD NPDAAHHHHHH

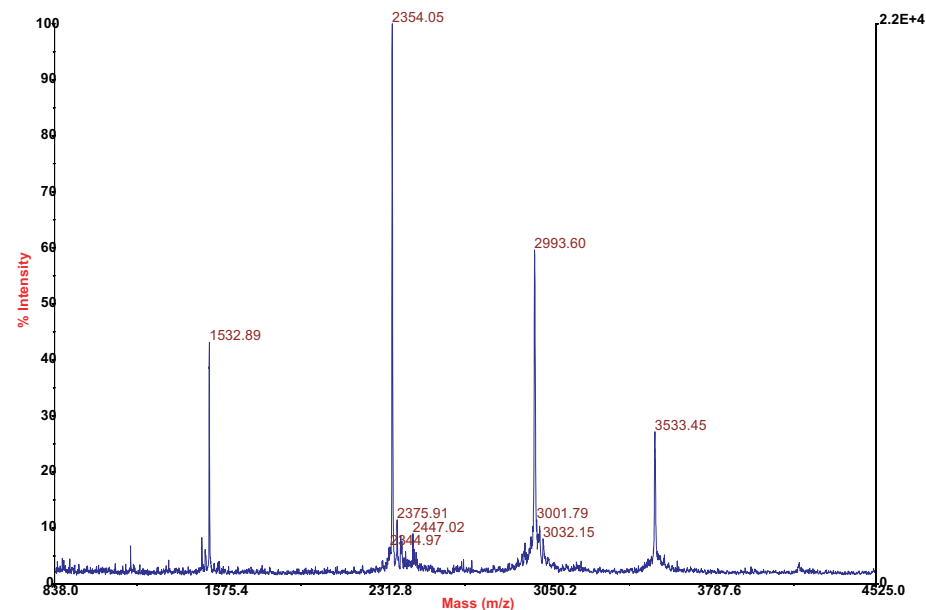
Notes: Glutamic acid residues are strongly favored by Endoproteinase GluC in all buffer conditions we have examined (Tris-HCl, ammonium bicarbonate and potassium phosphate) (2).

Endoproteinase GluC contains a 6-His-Tag on the C-terminal of the protein. The average protein appears as a single band on SDS-PAGE and a small amount of this protein may contain two extra Ala residues at the N-terminus of the protein (2). This sequence is also available at www.neb.com.

References:

1. Drapeau G. R., Boily, Y. and Houmard, J. (1972). *J. Biol. Chem.* 247, 6720–6726.
2. Benner, J. S., Martin, D. and Schampel, A. K., unpublished results.
3. Birktoft J. J. and Breddam, K. (1994). In A. J. Barrett (Ed.), *Methods Enzymol.* Vol. 244, (pp.114–126) San Diego: Academic Press.
4. Breddam K. and Meldal, M. (1992). *Eur. J. Biochem.* 206, 103–107.

MALDI-TOF MS: *Issatchenkia orientalis* Cytochrome c subjected to digestion by Endoproteinase GluC for 16 hours, dried and subjected to MALDI-TOF MS.



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181 IGIHWGGVPNEF NGAVFINENVR NFKQNI EDIHFANDD QPNNPDNPDN PNNPDNPNPD
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