Certificate of Analysis

Glu-C, Sequencing Grade:

Part No.	Size
V165A	50µg (5 × 10µg)

Description: Glu-C, Sequencing Grade (Cat.# V1651), is a serine protease that specifically cleaves at the C-terminus of either aspartic or glutamic acid residues (1–4). In ammonium bicarbonate and ammonium acetate, the enzyme specificity is higher at the glutamic residues. In phosphate buffers, cleavage occurs at the aspartic and glutamic residues (1,3,4). Glu-C activity is optimal in the pH range of 4.0–9.0. This sequencing grade enzyme can be used alone or in combination with other proteases to produce protein digests for peptide mapping applications or protein identification by peptide mass fingerprinting or MS/MS spectral matching. Glu-C, Sequencing Grade, is suitable for in-solution digestion reactions.

Biological Source: *S. aureus* V8.

Form: Lyophilized in the presence of trehalose (1.65mg/vial).

Molecular Weight: 27kDa.

Storage Conditions: Store at 4°C. See the Product Information Label for expiration date.

Usage Note: Reconstitute with filtered double-distilled water. Reconstituted enzyme solution can be stored at 4°C for 1 week or at –20°C for 8 weeks. Reconstituted enzyme can be frozen and thawed 5 times without a loss of activity.

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Quality Control Assays

This lot passes the following Quality Control specifications:

Activity: β-insulin substrate is digested at a ratio of 1:10 protease: substrate for 1 hour at 37°C. Reaction products are analyzed by HPLC. After 1 hour, all substrate is consumed and the expected Glu-C-dependent peaks are observed.

Specificity: No prominent nonspecific peaks are observed by reverse-phase HPLC analysis after 18 hours of incubation of β-insulin with Glu-C compared to the 1-hour digestion.

Usage Information on Back

References

1. Drapeau, G., Boily, Y. and Houmard, J. (1972) *J. Biol. Chem.* **247**, 6720–6.
2. Drapeau, G. (1978) *Can. J. Biochem.* **56**, 534–44.
3. Drapeau, G.R. (1977) *Meth. Enzymol.* **47**, 189–91.
4. Drapeau, G.R. (1976) *Meth. Enzymol.* **45**, 469–75.
5. Houmard, J. (1976) *Eur. J. Biochem.* **68**, 621–7.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. Preparation of Protein

In general, proteins require efficient solubilization, denaturation, disulphide bond reduction and alkylation for optimal digestion. The following optional steps are provided as a guideline to facilitate protease digestion with this product.

1. **Solubilization/Denaturation:** Dissolve protein in compatible buffer (Table 1). Proteins that are difficult to dissolve or require denaturation for efficient digestion can be solubilized in a minimal volume of denaturant, such as 6–8M urea or 6M guanidine HCl. Incubate at 20–37°C for up to 1 hour. Some proteins may benefit from heating the sample to 60°C for one hour, or 95°C for 15–20 minutes.
2. **Disulphide Reduction:** Add DTT (or β-mercaptoethanol) to a final concentration of 5mM. Heat to 50–60°C for 20 minutes.
3. **Alkylation:** Cool to room temperature. Add iodoacetamide to a final concentration of 15mM. Incubate in the dark at room temperature for 15 minutes.

   Adjust the reaction volume with buffer (pH 7.5) to reduce the final component concentrations for optimal digestion (Table 1).
4. **Digestion:** Add Glu-C, Sequencing Grade, to a final proteinase:protein ratio of 1:200 to 1:20 (w/w). Vortex to mix and centrifuge. Incubate sample at 37°C for 2–18 hours.

Table 1. Denaturant Compatibility and Reaction Conditions. The denaturing components listed were titrated into Glu-C reactions. Percent change of enzyme activity in the presence of the denaturants was determined by comparison to a reaction containing no denaturant. Denaturants were tested using a β-casein activity assay.

Component	Concentration	% Activity
Urea	0.4M 0.8M 1.4M	150 150 3
Guanidine HCl	0.18M 0.33M	100 57
SDS	0.004% 0.009% 0.016%	90 90 75
Acetonitrile	10% 20% 25% 30%	100 200 250 74

Additional Information	
Recommended Enzyme:Protein Ratio	1:20–200
Recommended Temperature	≤37°C
pH Range	4.0–9.0
Freeze-Thaw Cycles	5
Inhibitors	Diisopropyl fluorophosphate (DFP) Monovalent anions such as F^-, Cl^-, Br^-, CH_3COO^- and NO_3^- (1,5)

2. Related Products

Product	Size	Conc.	Cat.#
Asp-N, Sequencing Grade	2µg		V1621
Arg-C, Sequencing Grade	10µg		V1881
Chymotrypsin, Sequencing Grade	25µg		V1061
	100µg (4 × 25µg)		V1062
Elastase	5mg		V1891
Endo H	10,000u	500u/µl	V4871
	50,000u	500u/µl	V4875
Fetuin	500µg	10mg/ml	V4961
Immobilized Trypsin	2ml		V9012
	4ml (2 × 2ml)		V9013
Pepsin	250mg		V1959
PNGase F	500u	10u/µl	V4831
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg		V2071
	5 × 1mg		V2072
rLys-C, Mass Spec Grade	15µg		V1671
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Thermolysin	25mg		V4001
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073

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