

## Endoproteinase Glu-C from *Staphylococcus aureus* V8 suitable for protein sequencing

Catalog Number **P6181**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

CAS RN 66676-43-5  
EC 3.4.21.19  
Synonym: V8 Protease

### Product Description

Endoproteinase Glu-C from *Staphylococcus aureus* strain V8 is a serine endoprotease, which hydrolyzes peptide bonds at the carboxyl side of glutamyl and aspartyl residues. The specificity of Glu-C is dependent upon the buffer and pH employed as well as the structure around the potential cleavage site.<sup>1-7</sup> In ammonium acetate (pH 4.0) or ammonium bicarbonate (pH 7.8) the enzyme preferentially cleaves glutamyl bonds; whereas, in phosphate buffer (pH 7.8) Glu-C will cleave at either site. No cleavage will occur if a proline residue is on the carboxyl side.<sup>1</sup> The enzyme also exhibits esterase activity.<sup>1,2</sup>

Endoproteinase Glu-C is suitable for proteomic work. It is widely used in proteomics for peptide mapping and protein sequence work due to this highly specific cleavage of peptides resulting in a limited number of fragments.<sup>1-7</sup>

Endoproteinase Glu-C has an average molecular mass of 29.02 kDa.<sup>8,9</sup> The protease is active in the pH range of 3.5–9.5 and exhibits a double maximum of proteolytic activity at pH 4.0 and 7.8 with hemoglobin as the substrate.<sup>1,2</sup>

The specificity and activity of Glu-C is retained in 20% organic solvents.<sup>10</sup> Glu-C is reported to be fully active in the presence of 0.2% SDS and retains 50% of its activity in 4.0 M urea.<sup>2-6</sup> A known peptide such as the oxidized B chain of insulin should be run as a control for all experiments.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Reconstitute the lyophilized product in water.

### Storage/Stability

Store the product at  $-20\text{ }^{\circ}\text{C}$ . When stored at  $-20\text{ }^{\circ}\text{C}$ , the product retains activity for at least one year.

Frozen solutions can be thawed and frozen repeatedly without loss of activity.<sup>1</sup>

### Procedure

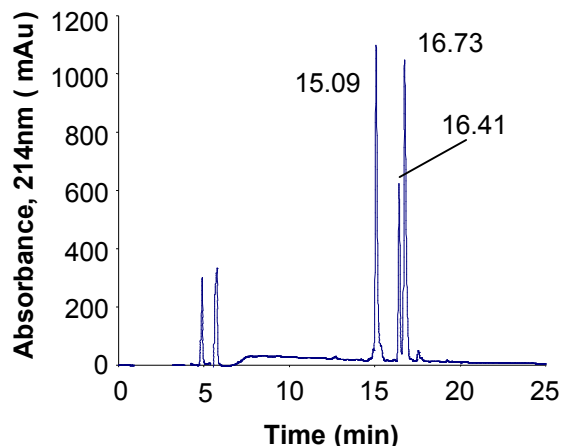
For peptide or protein digestion, a ratio between 1:20 and 1:100 (w/w) of enzyme to substrate is recommended. Dissolve the peptide or protein to be digested in 100 mM  $\text{NH}_4\text{HCO}_3$ , pH 7.8, or 100 mM Tris HCl, pH 7.8. Recommended incubation time is between 2–18 hours at  $37\text{ }^{\circ}\text{C}$  depending on the enzyme to substrate ratio. Endoproteinase Glu-C may also be used for in-gel digestions of proteins.<sup>11-14</sup>

### Results

The suitability of this product is demonstrated by the digestion of the oxidized B chain of insulin (Catalog Number I1764) as described in Figure 1. The sequence of the oxidized B chain of insulin is:

FVNQHLC<sub>ox</sub>GSHLVEALYLVC<sub>ox</sub>GERGFFYPKA

**Figure 1.**  
Suitability Assay of Glu-C



The oxidized B chain of insulin (100 µg) was digested with 5 µg of Glu-C for 18 hours at 37 °C in 100 µl of 100 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 7.8. A 20 µg aliquot was separated on a Discovery® C18 column (25 cm x 4.6 mm, 5 micron, Catalog Number 504971) using a 20 minute linear gradient from 5–50% B at 0.7 ml/min and was detected in the UV at 214 nm and by mass spectrometry. Solvent A: 0.1% (v/v) TFA in water, Solvent B: 0.08% (v/v) TFA in acetonitrile.

The Glu-C peptide fragments were identified as follows:

Retention Time (minutes)	Mass (Da)	Fragment
15.09	1529.4	Phe <sup>1</sup> – Glu <sup>13</sup>
16.41	914.3	Ala <sup>14</sup> – Glu <sup>21</sup>
16.73	1086.0	Arg <sup>22</sup> – Ala <sup>30</sup>

During the 18 hour digestion only the expected peptides were generated with no indication of other major proteolytic activity.

#### References

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#### Related Products suitable for protein sequencing

Product	Catalog Number
α-Chymotrypsin	C6423
Endoproteinase Arg-C	P6056
Endoproteinase Asp-N	P3303
Endoproteinase Lys-C	P3428
Leucine aminopeptidase	L9776
Trypsin	T8658
Insulin Chain B, Oxidized	I1764
Melittin	M1407
α-Melanocyte Stimulating Hormone	M4135

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