**Proteinase Sequence Grade**

Lysyl Endopeptidase, Endoproteinase Asp C, Endoproteinase Glu C

**Lysyl Endopeptidase, Mass Spectrometry Grade**
(Wako Catalog No. 125-05061 (5 x 20µg))

Among the most important techniques in proteome analyses is the in-gel digestion of protein spots/bands that have been resolved by electrophoresis using digestive enzymes, such as trypsin and lysyl endopeptidase. Proteins can be identified by mass spectrometry analysis of the peptides produced by in-gel digestion, and further information regarding post-translational modifications can be obtained. Lysyl Endopeptidase, Mass Spectrometry Grade is a freeze dried product that retained sufficient activity for in-gel digestion and packed in very small quantities for convenience purposes.

**Features**
1. High specificity and efficiency of protein digestion allow for easy database searches by peptide mass.
2. Improved cleavage at lysine residue and increase in the number of peptides are obtained by combination with trypsin.
3. Packed in very small quantities according to the amounts used so that sufficient activity for in-gel digestion may be retained.

**Comparison of In-gel Digestion Using Trypsin (Tp), Lysyl Endopeptidase (Lep) and Lep Combined with Tp (Lep +Tp)**

BSA band (100ng) resolved by SDS-PAGE was in-gel digested with Tp, Lep and Lep +Tp and analyzed by MALDI-TOFMS. The figure shows the individual mass spectra. The evaluation of these peptidases is summarized in the table.

**Table: Comparison of Tp, Lep and Lep +Tp**

<table>
<thead>
<tr>
<th>Cleavage site</th>
<th>Tp C terminal of Arg and Lys</th>
<th>Lep C terminal of Lys</th>
<th>Lep +Tp C terminal of Arg and Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missed cleavage (Rates of missed cleavage)*</td>
<td>Many (8%)</td>
<td>Very few (0%)</td>
<td>Few (3%)</td>
</tr>
<tr>
<td>No. of identified peptides</td>
<td>17</td>
<td>19</td>
<td>22</td>
</tr>
</tbody>
</table>

* The value resulted from subtracting the coverage obtained when database searches were performed with Missed cleavage 0 from that obtained when performed with Missed cleavage 1. "Coverage" is the percentage of peptides obtained after in-gel digestion in the whole sequence.

**References:**

**Related Products**
- **Negative Gel Stain MS Kit**
  - for Electrophoresis
  - Package Size: 20 tests
- **Silver Gel Stain MS Kit**
  - for Electrophoresis
  - Package Size: 20 tests

**Endoproteinase Asp-N, Sequencing grade**
- for Biochemistry
- Package Size: 2 µg

**Endoproteinase Glu-C, Sequencing grade**
- for Biochemistry
- Package Size: 50 µg

---

*Please visit the Wako Online Catalog to get further information: www.e-reagent.com*
Endoproteinase Asp N, Sequence grade (Wako Catalog No. 056-05921 (2 µg))

**Origin:** *Pseudomonas fragi*, mutant

**Preparation:** Lyophilized

**Specificity:** Cleave on the N-terminal side of Aspartic acid and Cysteic acid; after alkylation of Cys: only cleavage on Asp.

**Specific activity:** Indicated on each label (at 37°C with azocoll as substrate).

**Purity:** min. 90% (SDS-PAGE)

**Performance:** Cleavage after 1hr: min. 90% (with glucagon as substrate).

---

1. Asp (15)-Gln (20); 2. His (1)-Ser (8); 3. Asp (9)-Leu (14); 4. Asp (21)-Thr (29)

Digest: 100µg Glucagon + 10µg Endoproteinase Asp N Sequencing grade in 100µL Sodium Phosphate Buffer (50mmol/L, pH 8.0) 1h & 18h, 37°C

Sample: Digest 20µL

Chromato: Shandon ODS Hypersil, 5µm

Separation: Solvent A: Water (contains 0.1% TFA); Solvent B: Acetonitril : H₂O = 70:30 (contains 0.1% TFA)

Gradient: 20 min. linearly 0 – 100% B

Flow: 1 mL/min; Detection: 215 nm


---

Endoproteinase Glu C, Sequence grade (Wako Catalog No. 050-05941 (50 µg))

**Origin:** *Staphylococcus aureus* V8

**Preparation:** Lyophilized, salt-free

**Specificity:** In Ammonium carbonate buffer (pH 7.8) or ammonium acetate buffer (pH 4.0): Cleaves C-terminal peptide bonds of glutamic acid. In phosphate buffer (pH 7.8): Cleaves C-terminal peptide bonds of glutamic acid and aspartic acid.

**Specific activity:** Indicated on each label (at 25°C with Z-Phe-Leu-Glu-4-nitroanilide as substrate).

**Purity:** min. 90% (SDS-PAGE)

**Performance:** Cleavage after 1hr: min. 85% (in reverse phase HPLC, with insulin Box as substrate).

---