INSTRUCTION MANUAL

Endoproteinase Glu-C

Endoproteinase for proteomics

(Cat. No. 20986.01)

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1. General information

Endoproteinase Glu-C (V8) is a serine endoproteinase isolated from *Staphylococcus aureus V8*. Glu-C is suitable for proteomics applications.

The specificity of Glu-C is primarily determined by the buffer pH and composition. Using phosphate buffers (pH 7.8), Glu-C will cleave at both glutamyl and aspartyl bonds. Ammonium bicarbonate buffer (pH 7.8) will lead to a preferential cleavage of glutamyl bonds.

The presence of proline residues on the carboxy side of the peptide bond inhibits the cleavage.

Due to its highly specific cleavage of peptides Glu-C is used in proteomics for peptide mapping and protein sequence work.

1.1. Reconstitution of the enzyme

Dissolve the enzyme according to the protocols below.

1.2. Storage

Lyophilized enzyme can be stored at – 20 °C or – 80 °C.
Reconstituted enzyme can be stored at – 20 °C for 4 weeks.

2. Protocols

2.1. Optional

- Reduce the disulfide bonds of a protein sample by incubating the protein sample in a solution of 50 mM dithiothreitol (DTT) in 50 mM ammonium bicarbonate for 60 min at 55 °C.

- Alkylate the disulfide bonds of a protein sample by incubating the protein sample in a solution of 250 mM iodoacetamide / 50 mM ammonium bicarbonate for 60 min at room temperature in the dark.

2.2. In-solution digest

1. Reconstitute 1 vial of lyophilized enzyme in 250 µL of deionized water to make a 100 ng/µL solution.

2. Prepare a 5 ng/µL enzyme in Digestion Buffer solution by combining 5 µL of the 100 ng/µL enzyme solution (from Step 1.) with 95 µL 1x Digestion Buffer. The specific volumes and concentration can be adjusted for sample numbers and/or quantity of protein in the sample. A final enzyme to protein ratio of 1:20 to 1:100 is recommended.

3. Freeze any unused volume of reconstituted enzyme at -20 °C to -80 °C.

4. Combine the 5 ng/uL enzyme in Digestion Buffer solution (prepared in Step 2 above)
   with the protein sample (in 1x Digestion Buffer) at a 1:20 to 1:100 (w/w) enzyme to protein ratio. Mix gently.

5. Incubate at 37 °C for a duration between 2 h and overnight for digestion.

2.3. In-gel digest

1. Using a razor blade or hobby knife, cut the protein band of interest out of the gel and then dice the band into 1 mm x 1 mm pieces.

2. Rinse gel pieces with 50 µL of 1x Digestion Buffer.

3. Replace the Digestion Buffer with 50 µL acetonitrile and incubate at room temperature for 5 min to dehydrate the gel pieces.

4. Dry down gel pieces in SpeedVac (or lyophilizer).

5. Add 50 µL of 10 mM DTT in 1x Digestion Buffer and incubate samples for 1 h at 56 °C.

6. Cool to room temperature and using a micropipettor, replace DTT solution with 50 µL 250 mM iodoacetamide in 1x Digestion Buffer. Incubate samples at RT for 1 h.

7. Remove iodoacetamide solution with micropipettor.

8. Add 50 µL acetonitrile to gel pieces and incubate at room temperature for 5 min to dehydrate the gel pieces.

9. Remove acetonitrile with micropipettor and dry gel pieces in SpeedVac or lyophilizer.

10. Reconstitute the lyophilized enzyme in 250 µL of deionized water or 10 mM acetic acid to achieve a final concentration of 100 ng/µL. If only a small amount of enzyme is required for the experiment, aliquot the reconstituted enzyme into smaller volumes and immediately freeze and store at -20 to -80 °C.

11. Combine 10 µL of the 100 ng/µL reconstituted enzyme (prepared above, Step 10) with 90 µL of 1x Digestion Buffer to achieve a final concentration of 10.0 ng/µL). Place on ice until ready to use.

12. Add 50 µL of the 10 ng/µL enzyme solution (prepared above, Step 11) to the dried gel pieces and incubate on ice for 45 min.

13. Remove the 10 ng/µL enzyme solution from the gel pieces with a micropipettor and replace with 50 µL of 1x Digestion Buffer. Incubate overnight at 37 °C.

2.4. In-gel sample extraction

1. After performing in-gel digestion, remove the 1x Digestion Buffer from the gel pieces and collect in a clean vial (collection vial).

2. Add 50 µL of 1x Digestion Buffer to rinse the gel pieces. Remove the Digestion Buffer from the gel pieces and add it to the collection vial.

3. Add 50 µL of 50% acetonitrile / 5% formic acid solution to the gel pieces and incubate for 5 minutes at room temperature to dehydrate the gel pieces. Then, remove this solution from the gel pieces and add it to the collection vial.

4. Rehydrate the gel pieces by adding 50 µL of Digestion Buffer to the gel pieces and let incubate for 5 min. Then, remove the Digestion Buffer from the gel pieces and add it to the collection vial.

5. Repeat step 3.

6. Repeat step 4.

7. Repeat step 3.

8. Repeat step 4.

9. Repeat step 3.

10. Using a SpeedVac or lyophilizer, dry the collection vial and store at -20 to -80 °C for subsequent analysis.