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Product Information

Endoproteinase Asp-N from *Pseudomonas fragi* mutant strain
suitable for protein sequencing

Catalog Number **P3303**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

CAS RN 9001-92-7
EC 3.4.24.33
Synonym: Peptidyl-Asp-metalloendopeptidase

Product Description

Endoproteinase Asp-N is a metalloendoprotease, isolated from a mutant strain of *Pseudomonas fragi*. It hydrolyzes peptide bonds on the N-terminal side of aspartic and cysteic acid residues.[1-3] Endoproteinase Asp-N has an average molecular mass of 24.5 kDa, and a pH optimum between pH 6.0 and 8.5.[1]

Endoproteinase Asp-N is HPLC-purified, resulting in a product that is suitable for proteomic work. In 100 mM NH_4HCO_3, pH 8.5, or 100 mM Trizma® HCl, pH 8.5, Asp-N specifically cleaves peptide bonds on the N-terminal side of aspartic acid and cysteic acid (oxidized cysteine) residues.[1-3] Asp-N is used in proteomics for peptide mapping and protein sequence work, because of its highly specific cleavage of peptides, which results in a limited number of peptide fragments.[1-6]

Vial content: 2 µg of lyophilized Asp-N containing Trizma HCl.

Precautions and Disclaimer

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

Preparation Instructions

Reconstitute the lyophilized product in 50 µL of water. The protease will be in a solution containing 10 mM Trizma HCl, pH 8.0.

Storage/Stability

The lyophilized powder is stable for at least one year if stored desiccated at 2–8 °C. After reconstitution in water, frozen aliquots can be stored for several weeks.[1,3]

Procedure

For peptide or protein digestion, a ratio between 1:50 and 1:200 (w:w) of enzyme to substrate is recommended. Dissolve the peptide or protein to be digested in 100 mM NH_4HCO_3, pH 8.5, or in 100 mM Trizma HCl pH 8.5. The recommended incubation time is between 2 and 18 hours at 37 °C, depending on the enzyme to substrate ratio. Endoproteinase Asp-N may also be used for in-gel digestions of proteins.[7-10]

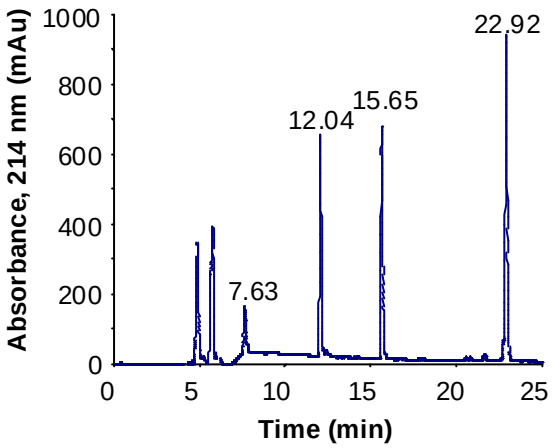
Self-digestion may occur if temperatures >37 °C are used. Asp-N retains most of its activity in 2.0 M urea, 1.0 M guanidine HCl. or 0.1% SDS.[1,6] A known peptide such as glucagon should be run as a control for all experiments.

Results

The suitability of this product is demonstrated by digestion of glucagon, as described in Figure 1. The sequence of glucagon is:

HSQGTFTSDYSKYLDSRRAQDFVQWLMNT

Figure 1.
Suitability Assay of Asp-N



Glucagon (40 μg) was digested with 2 μg of Asp-N for 18 hours at 37 °C in 100 mM NH_4HCO_3, pH 8.5. A 20 μg aliquot was separated on a Supelco Discovery C_{18} column (25 cm × 4.6 mm, 5 micron, Catalog Number 504971) using a 20 minute linear gradient from 5-50% B at 0.7 mL/min with UV detection 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 Asp-N proteolytic fragments were identified as follows:

Retention Time (min)	Mass (Da)	Fragment
7.63	731.3	Asp^{15}–Gln^{20}
12.04	863.3	His^{1}–Ser^{8}
15.65	787.3	Asp^{9}–Leu^{14}
22.92	1,152.3	Asp^{21}–Thr^{29}

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

References

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