Print

Endoproteinase Asp-N

Sequencing Grade
From *Pseudomonas fragi* mutant

Cat. No. 11 420 488 001 2 µg
Cat. No. 11 054 589 001 3 × 2 µg

Version 17
Content version: March 2016

Store at +2 to +8°C

1. What this Product Does

Content
Lyophilizate

Storage and Stability
The lyophilizate is stable at +2 to +8°C until the expiration date printed on the label. The working solution of Endoproteinase Asp-N in double-distilled water may be used for maximum of one week when stored at +2 to +8°C.

Store dry!

Application
Use Endoproteinase Asp-N for protein structure analysis and sequence analysis.

2. How to Use this Product

2.1 Before You Begin

General Handling Recommendations
The content of one vial may be used for several simultaneous digests. A new vial should be taken when repeating a digest in order to minimize the risk of contamination or autolysis.

2.2 Digestion of Proteins in Solution

Working Solution
Reconstitute lyophilized Endoproteinase Asp-N in 50 µl double-distilled water to give a final concentration of 10 mM Tris-HCl buffer, pH 7.5.

In order to avoid autolysis, the incubation temperature should not exceed +37°C.

Procedure

Step	Action
1	Dissolve the proteins to be sequenced in digestion buffer (50 mM sodium phosphate buffer, pH 8.0).
2	In the case of proteins that are hard to solubilize, add urea, SDS, or guanidine hydrochloride to the digestion buffer prior to solubilizing the protein. When applying urea, Roche recommends also adding 20 mM methylamine.
3	To achieve a suitable concentration of the denaturing agent in the digest, the protein solution should be correspondingly diluted with buffer (Tab. 1). Using complexing agents (such as EDTA or 2-phenanthroline) in the samples should be avoided, because Endoproteinase Asp-N is a metallo protease.
4	The recommended amount of enzyme is 1/200 to 1/20 of the protein by weight.
5	The incubation time should be chosen between 2 and 18 h at +37°C depending on the amount of enzyme. Under these conditions, additional cleavage at glutamyl residues can be observed. Recent investigations (3) on the cleveage velocity of Endoproteinase Asp-N revealed that the aspartyl specific cleavage is at least 2,000-fold faster than the glutamyl side activity of the Endoproteinase Asp-N. The additional cleavage at glutamyl residues can be prevented by reducing the enzyme concentration (enzyme-substrate-ratio of 1:1000, [w/w]) at an incubation time of 2 to 6 h.

Tab. 1: Activity determination of Endoproteinase Asp-N, with azocoll as substrate in the presence of stated concentrations of denaturing agents. Incubation of Endoproteinase Asp-N 200 µg/ml, with denaturing agent for 6 h at 25°C in 25 mM sodium phosphate buffer, pH 7.8.

Roche recommends also adding 20 mM methylamine when applying urea.

Denaturing agent	Concentration	Enzyme activity in %
without addition (control)	-	100
sodium dodecyl sulfate (SDS)	0.001% (w/v) 0.01% (w/v) 0.1% (w/v)	113 122 10
urea	0.1 M 0.5 M 1.0 M	100 108 105
guanidine hydrochloride	0.1 M 0.5 M 1.0 M	100 85 80
acetonitrile	1% (v/v) 5% (v/v) 10% (v/v)	90 115 125

3. Additional Information on this Product

3.1 Product Characteristics

Molecular Weight

27 kDa

Source

The wild type of *Pseudomonas fragi* segregates a metallo protease, which cleaves peptide bonds N-terminally at small hydrophilic amino acids (1). A mutant of this strain produces the Endoproteinase Asp-N, which is isolated as a highly purified and specific protease.

Sequence of Endoproteinase Asp-N

Partial sequences of Endoproteinase Asp-N are described (4, 5).

3.2 Quality Control

Function and purity control by HPLC of each lot ensure constant quality.

According to the current quality control procedures, the enzyme is free of impurities that might interfere with the separation range of peptides in reversed-phase HPLC (highly sensitive detection at 206-230 nm).

Specificity and Nonspecificity Verification

Endoproteinase Asp-N is a metallo protease that specifically cleaves peptide bonds N-terminally at aspartic and cysteic acids in phosphate, acetate or Tris buffers at pH 6.0-8.5 (2).

The specificity and nonspecificity of Endoproteinase Asp-N is verified with glucagon or melittin as substrate.

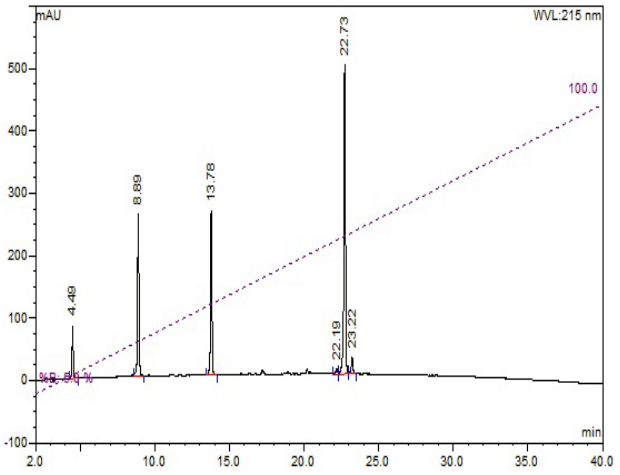

Fig. 1: Specificity of Endoproteinase Asp-N in reversed phase HPLC. High concentrations of Endoproteinase Asp-N (1 part by weight enzyme with 100 parts by weight glucagon) are incubated for 1 h to detect the fragments of the specific digested substrate.

Digest	40 µg glucagon in 200 µl 45 mM NaH_2PO_4 buffer, 0.09% TFA at pH 8.0; + 0.4 µg Endoproteinase Asp-N dissolved in 10 µl water; 1 h at +37 °C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength:	215 nm
Fragments	4.49 min Asp (15) - Gln (20) 8.89 min His (1) - Ser (8) 13.78 min Asp (9) - Leu (14) 22.73 min Asp (21) - Thr (29)

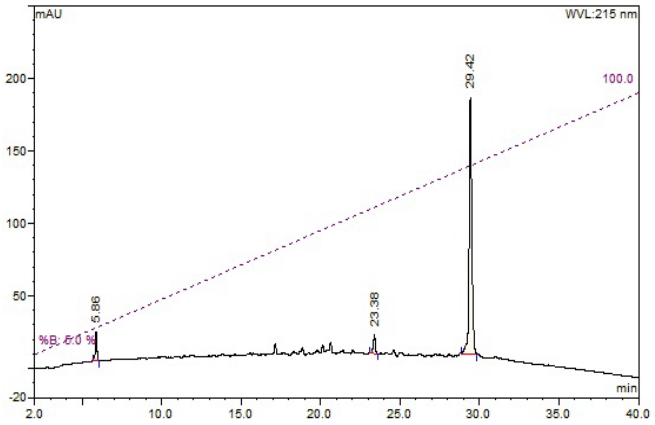

Fig. 2: Nonspecificity of Endoproteinase Asp-N in reversed phase HPLC. High concentrations of Endoproteinase Asp-N (1 part by weight enzyme with 20 parts by weight melittin) are incubated for 4 h to detect traces of impurities.

Digest	40 µg melittin in 200 µl 50 mM NaH_2PO_4 buffer, at pH 8.0; + 2 µg Endoproteinase Asp-N dissolved in 50 µl water, 4 h at +37 °C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B
Flow rate	1 ml/min
Wavelength:	215 nm

References

1 Noreau, J. & Drapeau, G. R. (1979) *J. Bacteriol.* **140**, 911-916
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3 Deuß, U. *et al.* (1990) *J. Prot. Chem.* **9**, 299-300
4 Hagmann, M.-L. *et al.* (1995) *Methods Enzymol.* **248**, 782-787
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4. Supplementary Information

Changes to Previous Version

- Editorial Changes

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ❶, ❷, etc.	Stages in a process that usually occur in the order listed.

Symbols

Symbols are used in this document to highlight important information:

Symbol	Description
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