For life science research only.
Not for use in diagnostic procedures.

Endoproteinase Asp-N Sequencing Grade
from *Pseudomonas fragi* mutant

Version: 18
Content Version: June 2021

Lyophilized

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

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Endoproteinase Asp-N Sequencing Grade	Highly purified and specific protease.	11 420 488 001	1 vial, 2 µg
			11 054 589 001	3 vials, 2 µg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Endoproteinase Asp-N Sequencing Grade	Store at +2 to +8°C. ⚠ ***Store dry.***

1.3. Additional Equipment and Reagent required

For preparation of digestion buffer

See section, **Working Solution** *for additional information on preparing solutions.*

- Sodium phosphate buffer

For solubilization of proteins

- Sodium dodecyl sulfate (SDS*)
- Urea
- Methylamine
- Guanidine hydrochloride
- Acetonitrile

1.4. Application

Use Endoproteinase Asp-N for the specific cleavage of proteins and peptides for:

- Protein structure
- Sequence analysis

2. How to Use this Product

2.1. Before you Begin

General Considerations

General handling recommendations

The content of one vial may be used for several simultaneous digests.
⚠ ***Take a new vial when repeating a digest in order to minimize the risk of contamination or autolysis.***

Activity determination

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 hours at +25°C in 25 mM sodium phosphate buffer, pH 7.8.
ⓘ *Add 20 mM methylamine when applying urea.*

Denaturing agent	Concentration	Enzyme activity [%]
without addition (control)	–	100
SDS	0.001% (w/v) 0.01% (w/v) 0.1% (w/v)	113 122 10
Urea (+ methylamine)	0.1 M 0.5 M 1 M	100 108 105
Guanidine hydrochloride	0.1 M 0.5 M 1 M	100 85 80
Acetonitrile	1% (v/v) 5% (v/v) 10% (v/v)	90 115 125

Working Solution

Solution	Preparation/Composition	Storage and Stability	For use in...
Endoproteinase Asp-N Sequencing Grade	▪ Add 50 µl double-distilled water to the lyophilizate to a final concentration of 10 mM Tris-HCl, pH 7.5. ⚠ ***To avoid autolysis, the incubation temperature must not exceed +37°C.***	Store 1 week at +2 to +8°C.	Digestion mixture
Digestion buffer	50 mM sodium phosphate buffer, pH 8.0.	–	Dissolution of the proteins to be sequenced.

2.2. Protocols

Digestion of proteins in solution

See section, **Working Solution** *for information on preparing solutions.*

1. Dissolve the proteins to be sequenced in Digestion buffer.
   For proteins that are hard to solubilize, add urea, SDS, or guanidine hydrochloride to the Digestion buffer prior to solubilizing the protein. When applying urea, also add 20 mM methylamine.

2. Dilute protein solution with buffer, see section, **General Considerations** to achieve a suitable concentration of the denaturing agent in the digest.
   Avoid complexing agents, such as EDTA or 2-phenanthroline in the samples, since Endoproteinase Asp-N is a metalloprotease.
   The recommended amount of enzyme is 1/200 to 1/20 of the protein by weight.

3. Choose an incubation time between 2 and 18 hours at +37°C, depending on the amount of enzyme.
   Under these conditions, additional cleavage at glutamyl residues can be observed. The cleavage 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.
   Reduce enzyme concentration (enzyme-substrate ratio of 1:1,000 [w/w]) at an incubation time of 2 to 6 hours to prevent the additional cleavage at glutamyl residues.

2.3. Parameters

Molecular Weight

27 kDa

3. Results

Verification of specificity and nonspecificity of Endoproteinase Asp-N

Endoproteinase Asp-N is a metalloprotease that specifically cleaves peptide bonds N-terminally at aspartic and cysteic acids in phosphate, acetate, or Tris buffers at pH 6.0 to 8.5.
The specificity and nonspecificity of Endoproteinase Asp-N is verified with glucagon or melittin as substrate.

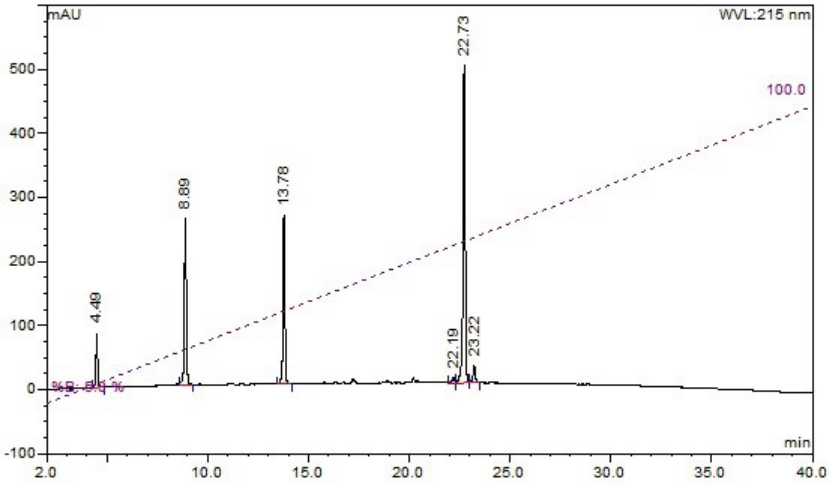

Fig 1: Specificity of Endoproteinase Asp-N in reversed phase HPLC. High concentrations of Endoproteinase Asp-N (1 part by weight enzyme with 100 parts by weight glucagon) are incubated for 1 hour to detect the fragments of the specific digested substrate.

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 double-distilled water; 1 hour at +37°C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
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)

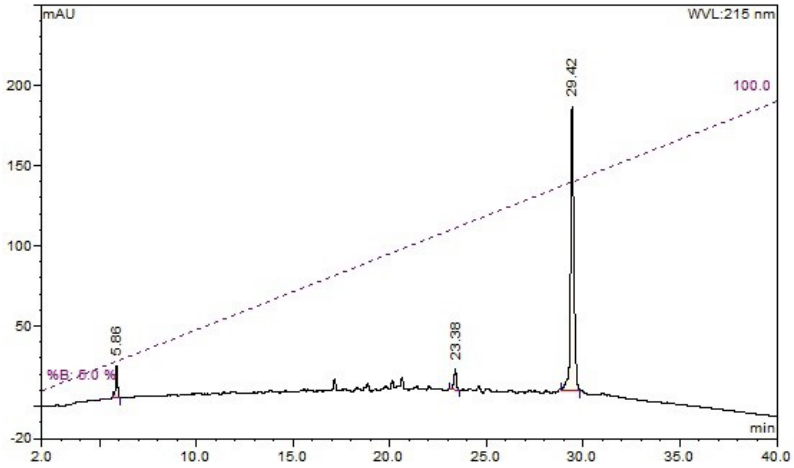

Fig 2: Nonspecificity of Endoproteinase Asp-N in reversed phase HPLC. High concentrations of Endoproteinase Asp-N (1 part by weight enzyme with 20 parts by weight melittin) are incubated for 4 hours to detect traces of impurities.

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 double-distilled water; 4 hours at +37°C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
Wavelength	215 nm

4. Additional Information on this Product

4.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

0621.11056573001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany