Endoproteinase AspN

1-800-632-7799
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www.neb.com

P8104S 002160617061

P8104S

50 µg **Lot: 0021606**

Store at –20°C **Exp: 6/17**

Description: Endoproteinase AspN (flavastacin) is a zinc metalloendopeptidase which selectively cleaves peptide bonds N-terminal to aspartic acid residues (1).

Source: Purified from *Flavobacterium menigosepticum.*

Applications:
- Digestion of peptides of 5 kDa or less for proteomic analysis by Mass Spectrometry
- Protein and peptide identification

Reaction Conditions: 1X AspN Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X AspN Reaction Buffer.

1X AspN Reaction Buffer:
50 mM Tris-HCl
2.5 mM Zinc Sulfate
pH 8.0 @ 37°C

Specific Activity: ~ 25 µmol/min/mg

Molecular Weight: 40,089.9 daltons

Reconstitution: Endoproteinase AspN should be reconstituted by the addition of 50–500 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Storage Conditions: Supplied in lyophilized form.

Can be stored frozen in solution at –20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

Quality Assurance: Endoproteinase AspN is free of glycerol and detergents which may interfere with Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) or Electrospray Ionization (ESI) Mass Spectrometry (MS), or liquid chromatography (LC) methods.

Quality Controls

Endoproteinase AspN Activity:
Measured in two assays:

1. Peptide digestion and analysis by MALDI-TOF MS or ESI-TOF MS
2. Fluorometric substrate digestion and specific activity determination in digestion buffer: 50 mM Tris-HCl (pH 8.0) and 2.0 mM Zinc Acetate.

Peptide Digestion: ACTH (18–39) peptide is subjected to digestion by AspN at a ratio of 20:1 respectively for 16 hours at 37°C in AspN Reaction Buffer. 1 µl of the above reaction (10 ng) was mixed with 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution, air-dried and subjected to MALDI-TOF MS analysis.

Fluorometric Assay: 1 µg (~1 µmol) of Anthranilyl-Ala-Phe-Ala-Phe-Asp-Val-Phe(NO_2)-Tyr-Asp peptide (Sigma) was suspended in 150 µl of AspN Reaction Buffer and 1 µg of Endoproteinase AspN was added (4). The initial rate was determined by measurement of the increase in fluorescence (excitation 330 nm and emission 450 nm). The protein concentration is determined by C18 reverse-phase HPLC and integration.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Endoproteinase AspN Protein Sequence:

```
  1 TIVSSFIKTWPNATVYYTLPSQGSLSTQAYNTFLTNINKAFDMISSKTSVKFVQRTNQTE
 61 YITFTYSTGNSSPLGWVKNRVNGIKIYNTTYPAIIAHEIMHSMGIMHEQCRPDRDQYIIV
121 DTNRAQDGTRHNFNLYNDYAGHGEFDFGSVMMYKSTDFAIDPNLPVMTKLDGSTFGKQRD
181 GLSAGDYAGINHLYGPVNSTSATNGTYTLTTSLAGDKNIDITGSSTADGTDVILYSATTG
241 NNQKFIFRKSEHGYFTIKSILDSTKVLTVRNNGTANGTAVELRTNADTDAQKWLLFNLGN
301 EGFGFAPKNAPSLRLEVKDGLTTNLTPIVIGSTDQTLQPYTKQRFTLTKVN
```

Notes: Aspartic acid residues are strongly favored by Endoproteinase AspN in all buffer conditions we have examined (Tris-HCl, ammonium bicarbonate and potassium phosphate) (2).

Endoproteinase AspN is recommended for cleavage of peptides only. The cleavage rate of protein is much slower

Endoproteinase AspN contains a O-linked carbohydrate on the protein. The protein appears as a single band by SDS-PAGE analysis. The protein sequence is also available at www.neb.com.

References:

1. Tarentino A.L. Flavastacin. In Handbook of Proteolytic Enzymes, 2nd Ed. (Barret, A.J., Rawlings, N.D. and Woessner, J.F. eds.) P. 631–632, Elsevier, London (2004).
2. Tarentino, A.L. et al. (1995) *Arch Biochem Biophys.* 319, 281–285.
3. Grimwood, B.G., Plummer, T.H. and Tarentino, A.L. (1994) *Arch Biochem Biophys.* 311, 127–132.

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  1 TIVSSFIKTWPNATVYYTLPSQGSLSTQAYNTFLTNINKAFDMISSKTSVKFVQRTNQTE
 61 YITFTYSTGNSSPLGWVKNRVNGIKIYNTTYPAIIAHEIMHSMGIMHEQCRPDRDQYIIV
121 DTNRAQDGTRHNFNLYNDYAGHGEFDFGSVMMYKSTDFAIDPNLPVMTKLDGSTFGKQRD
181 GLSAGDYAGINHLYGPVNSTSATNGTYTLTTSLAGDKNIDITGSSTADGTDVILYSATTG
241 NNQKFIFRKSEHGYFTIKSILDSTKVLTVRNNGTANGTAVELRTNADTDAQKWLLFNLGN
301 EGFGFAPKNAPSLRLEVKDGLTTNLTPIVIGSTDQTLQPYTKQRFTLTKVN
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Page 2 (P8104)

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