Certificate of Analysis

Asp-N, Sequencing Grade:

Part# 9PIV162

Revised 11/20

Part No.	Size
V162A	2µg

Description: Asp-N, Sequencing Grade (Cat.# V1621), is an endoproteinase that hydrolyzes peptide bonds on the N-terminal side of aspartic, and to a lesser extent, glutamic acid residues (Asp and Glu) (1,2).

Biological Source: *Pseudomonas fragi.*

Form: Lyophilized.

Molecular Weight: 24.5kDa.

Storage Conditions: Store at 4°C. See the Product Information Label for expiration date.

Usage Note: Asp-N, Sequencing Grade, is lyophilized in the presence of Tris-HCl. When reconstituted in 50µl of double-distilled water, the protease solution contains 10mM Tris-HCl (pH 8.0). Resuspended Asp-N, Sequencing Grade, can be stored at 4°C for up to 1 week or frozen for several weeks.

AF9PIV162 1120V162

Quality Control Assays

This lot passes the following Quality Control specifications:

Purity: Purity by SDS-PAGE is ≥98%.

Usage Information on Back

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References

1. Ingrosso, D. (1989) *Biochem. Biophys. Res. Comm.* **162**,1528–34.
2. Drapeau, G.R. (1980) *J. Biol. Chem.* **255**, 839–40.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. In-Solution Digestion Protocol

Preparation of Protein

In general, proteins require efficient solubilization, denaturation, disulphide bond reduction and alkylation for optimal digestion. The following optional steps are provided as a guideline to facilitate protease digestion with this product.

1. **Solubilization/Denaturation:** Dissolve protein in compatible buffer (Table 1). Proteins that are difficult to dissolve or require denaturation for efficient digestion can be solubilized in a minimal volume of denaturant, such as 6–8M urea or 6M guanidine HCl, at room temperature to 37°C for up to 1 hour. Some proteins may benefit from heating the sample to 60°C for 1 hour, or 95°C for 15–20 minutes. ProteaseMAX™ Surfactant can be used (0.01–0.1%) in compatible buffer (pH 7.5) in a minimal volume and does not require heating to be effective. A high concentration of denaturants may reduce the activity of the enzyme (see Table 1).
2. **Disulphide Reduction:** Add DTT (or β-mercaptoethanol) (final concentration of 5mM). Heat to 50–60°C for 20 minutes.
3. **Alkylation:** Cool to room temperature. Add iodoacetamide to a final concentration of 15mM. Incubate in the dark at room temperature for 15 minutes.

   Adjust the reaction volume with buffer (pH 7.5) to reduce the final component concentrations for optimal digestion (see Table 1).
4. **Digestion:** Add Asp-N, Sequencing Grade, to a final protease:protein ratio of 1:200 to 1:20. (w/w). Vortex to mix and centrifuge. Incubate 2–18 hours at 37°C.

2. In-Gel Digestion Protocol

(See also the *Trypsin Gold, Mass Spectrometry Grade Technical Bulletin*, #TB309, for another example of an in-gel digestion protocol and C18 cleanup.)

Preparation of the Protein Sample

1. Resolve proteins by gel electrophoresis, then stain the gel. Wash with an appropriate destaining solution to remove nonspecifically bound stain. In the example below, SimplyBlue™ SafeStain was used. Other gel systems and staining reagents can be used for in-gel digestions but should be tested to ensure compatibility with mass spectrometry analysis.
2. Excise the protein band, cut into 1mm^3 pieces and transfer to a 1.5ml microcentrifuge tube. Perform the following steps at room temperature.
3. Wash with 200µl of double-distilled water. Vortex, and then discard the wash solution. Repeat 2 times for a total of three washes.
4. Destain with 200µl of 50% acetonitrile (methanol can be substituted). Vortex. Incubate for 10 minutes, and then discard the wash solution.
5. Add 200µl of double-distilled water. Vortex, incubate 1 minute, discard wash solution.
6. Dehydrate the gel in 100µl of 100% acetonitrile. Vortex, incubate for 1 minute, and then discard the solution. Dry in a speed vacuum for 5–10 minutes.
7. Add 100µl of 25mM DTT in 50mM Tris HCl. Vortex, and then place in 56°C water bath. Incubate for 20 minutes, and then discard the solution.
8. Add 100µl of 55mM of iodoacetamide. Incubate in the dark at room temperature for 20 minutes. Discard the solution.
9. Wash with 200µl of double-distilled water. Vortex, and then discard the wash solution. Repeat 2 times for a total of three washes.
10. Add 100µl of 50% acetonitrile. Vortex, incubate for 10 minutes, discard solution.
11. Add 100µl of 100% acetonitrile to the gel. Vortex, and then incubate for 1 minute. Discard the solution. Dry in a speed vacuum for 5–10 minutes.
12. Reconstitute 2µg of Asp-N with double-distilled water. Add to gel slice in ratio of 1:50 enzyme to protein, in a total volume of 100µl. Vortex, and then incubate in a 37°C incubator overnight. Centrifuge, collect and transfer the supernantant to a new 1.5ml microcentrifuge tube.
13. Add 100µl of 50% acetonitrile solution. Vortex, and then incubate for 15 minutes.
14. Add 100µl of 20% formic acid to the gel. Vortex, and then incubate for 25 minutes.
15. Add 100µl of 100% acetonitrile solution. Vortex, and then incubate for 15 minutes.
16. Centrifuge and collect the supernatant. Combine with the supernatant from Step 12.
17. Dry the combined supernatants (Step 16) in a speed vacuum (1–3 hours).
18. Reconstitute and analyze samples. Sample cleanup prior to analysis may be necessary using a product such as Millipore C18. The need for cleanup depends on the method of analysis.

Table 1. Components Titrated into Reactions. Components listed were tested by titration into an Asp-N digestion reaction containing a fluorescent substrate (Anthraniloyl-Ala-Phe-Ala-Phe-Asp-Val-Phe-3-nitro-Tyr-Asp-OH). Inhibition results were confirmed by HPLC analysis of Asp-N digests.

Component	Concentration	% Activity
Urea	3.5M 4.5M 8M	100 80 30
Guanidine HCl	1M 2M 3M	100 60 16
SDS	0.028% 0.05%	100 85
ProteaseMAX™ Surfactant	0.026%	95
Acetonitrile	20% 40% 60% 80%	100 100 100 90
EDTA	2.0mM 5.6mM 10mM	100 80 70
Additional Information		
Recommended Enzyme:Protein Ratio		1:20–200
Recommended Temperature		≤37°C
pH Range		4.0–9.0
Freeze-Thaw Cycles		5
Buffer Compatibility		Tris-HCl, $NaPO_4$, Ammonium Bicarbonate

3. Related Products

Product	Size	Conc.	Cat.#
Arg-C, Sequencing Grade	10µg		V1881
Chymotrypsin, Sequencing Grade	25µg		V1061
	100µg (4 × 25µg)		V1062
Elastase	5mg		V1891
Endo H	10,000u	500u/µl	V4871
	50,000u	500u/µl	V4875
Glu-C, Sequencing Grade	50µg (5 × 10µg)		V1651
Immobilized Trypsin	2ml		V9012
	4ml (2 × 2ml)		V9013
Pepsin	250mg		V1959
PNGase F	500u	10u/µl	V4831
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg		V2071
	5 × 1mg		V2072
rLys-C, Mass Spec Grade	15µg		V1671
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Thermolysin	25mg		V4001
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073

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