Certificate of Analysis

rAsp-N, Mass Spec Grade:

Part No.	Size
VA116A	10µg

Description: rAsp-N, Mass Spec Grade (Cat.# VA1160), is an endoproteinase that hydrolyzes peptide bonds primarily on the N-terminal side of aspartic acid residues. Cleavage on the N-terminal side of glutamic acid residues can occur at a slower rate.

Biological Source: rAsp-N, Mass Spec Grade, was cloned from *Stenotrophomonas maltophilia.* rAsp-N is expressed in and purified from *E. coli.*

Expiration Date: See the Product Information Label for expiration date.

Form: Lyophilized.

Molecular Weight: Approximately 25kDa.

Storage Conditions: Store the lyophilized product at –30°C to –10°C.

Usage Note: rAsp-N, Mass Spec Grade, is lyophilized in Tris (pH 8.0) with NaCl and stabilizing sugars. Reconstitute in 50µl of ultrapure water and mix gently. Reconstituted rAsp-N can be stored at 4°C for at least 8 weeks. For longer storage, single-use aliquots can be stored at –65°C or below. Avoid freeze-thaw cycles.

Quality Control Assays

This lot passes the following Quality Control specifications:

Purity: Purity by SDS-PAGE is ≥90%.

Activity by HPLC: ≥90% of Cecropin A is digested by rAsp-N at an enzyme:substrate ratio of 1:1,000, when incubated at 30°C for 15 minutes.

Mass Spec Identification of Digested Monoclonal Antibody: NISTmAb is denatured, reduced, alkylated, desalted and then digested with rAsp-N, Mass Spec Grade, for 2 hours at 37°C at a 1:10 enzyme:substrate ratio. ≥50% sequence coverage of both heavy and light chains of NISTmAb is obtained.

Usage Information on Back

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIVA116

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Promega

Promega Corporation

2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Telephone 608-274-4330
Toll Free 800-356-9526
Fax 608-277-2516
Internet www.promega.com

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Usage Information

Reconstitution of rAsp-N, Mass Spec Grade

Reconstitute rAsp-N, Mass Spec Grade, by adding 50µl of water and mixing gently. Store on ice or at 4°C.

Before You Begin

The following protocols serve only as guidelines, since digestion conditions vary depending on the objective of the experiment. Typical variables to optimize include:

- Enzyme-to-substrate ratio: 1:10 to 1:100.
- Digestion time: 1–18 hours. Increased proteolysis at glutamic acid residues may be observed as digestion times increase.
- Buffer pH: rAsp-N has maximal activity at pH 8, but pH buffers ranging from 6–9 can be used. Increase digestion times accordingly.
- Denaturants: rAsp-N has been successfully tested in up to 2M urea. If guanidine-HCl is used as a denaturant, we recommend removing it through buffer exchange prior to digestion.
- Reducing agent and alkylating agent concentrations, such as DTT and iodoacetamide. Alternatively, evaluate reagents such as TCEP and iodoacetate. **Important**: Do not quench unreacted alkylating agent with excess DTT. Excess DTT will inhibit enzyme activity. If alkylating agent must be removed, we recommend using a desalting column.

Note: rAsp-N has a histidine tag that can be used to remove it from solution.

Protocol for Digestion of Complex Mixtures

The following protocol is an example of the use of rAsp-N to digest a complex protein mixture such as yeast extract.

1. Reconstitute rAsp-N, Mass Spec Grade, as instructed at the top of this page.
2. Thaw one vial of yeast extract (Cat.# V7341, 1mg at 10µg/µl in 6.5M urea) and reduce by addition of 1µl of 0.5M DTT (final concentration 5mM). Incubate at 37°C for 30 minutes. **Note:** Avoid temperatures higher than 37°C due to the presence of urea. Higher temperatures can cause carbamylation.
3. Alkylate by addition of 1.5µl of 1M iodoacetamide (IAM) (final concentration of 15mM) and incubate in the dark at room temperature for 1 hour.
4. To a digestion tube add: 25µg (2.5µl) of yeast extract and 4–8 volumes of a digestion buffer such as 50mM Tris (pH 8) or 50mM ammonium bicarbonate.
5. Initiate digestion by addition of 0.5µg (2.5µl) of rAsp-N for a final enzyme:substrate ratio of 1:50.
6. Incubate at 37°C for 60 minutes.
7. Terminate the reaction by acidification with TFA or formic acid (typically ~0.1–1% final concentration or until a pH of ~2–3 is reached).
8. Prior to LC-MS analysis, desalt the digested peptides either with offline SPE or with an inline trap column.

Protocol for IgG Digestion

The following protocol is an example of the use of rAsp-N, Mass Spec Grade, to digest proteins such as IgG that may be resistant to proteolytic digestion unless fully denatured, reduced and alkylated.

1. Reconstitute rAsp-N, Mass Spec Grade, as instructed at the top of this page.
2. Denature IgG in 6M guanidine-HCl as follows:
   a. For example, use 25µl of 10µg/µl IgG (250µg).
   b. Add 75µl of 8M guanidine HCl (6M final concentration).
   c. Mix and incubate for 15 minutes at 37°C.
3. Reduce by adding DTT to 10mM and incubating for 30 minutes at 37°C. For example, add 1µl of 1M DTT.
4. Alkylate by adding iodoacetamide (IAM) to 30mM and incubating for 30 minutes at room temperature in the dark. For example, add 6.5µl of 0.5M IAM.
5. Remove the denaturant, reducing and alkylating agents with a spin-desalting column such as a Zeba™ or Bio-Spin® column. Suggested digestion buffers include 50mM Tris (pH 8) or 50mM ammonium bicarbonate.
6. Add rAsp-N, Mass Spec Grade, at the desired enzyme:substrate ratio and incubate at 37°C. For example, at a 1:10 ratio, digestion may be complete in approximately 1–2 hours. Alternatively, a 1:50 ratio may require overnight incubation.
7. Terminate the reaction by acidification using TFA or formic acid (typically ~ 0.1–1% final concentration or until a pH of ~2–3 is reached) and proceed to LC-MS.

Related Products

Product	Size	Conc.	Cat.#
Asp-N, Sequencing Grade	2µg		V1621
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
IdeS Protease (lyophilized)	5,000 units		V7511
	25,000 units (5 × 5,000 units)		V7515
IdeZ Protease (lyophilized)	5,000 units		V8341
	25,000 units (5 × 5,000 units)		V8345

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