

Certificate of Analysis

Thermolysin:

Part No.	Size
V400A	25mg

Description: Thermolysin is a thermostable metalloproteinase. The high digestion temperatures may be used as an alternative to denaturants to improve digestion of proteolytically resistant proteins. Thermolysin preferentially cleaves at the N-terminus of the hydrophobic residues leucine, phenylalanine, valine, isoleucine, alanine and methionine. This enzyme can be used alone or in combination with other proteases for protein analysis by mass spectrometry, protein structural studies and other applications.

Biological Source: *Geobacillus stearothermophilus*.

Molecular Weight: 36.2kDa (1).

Form: Lyophilized.

Storage Conditions: See the Product Information Label for storage conditions and expiration date.

Optimal pH: 8.0. Thermolysin is stable from pH 5.0–8.5 (2).

Activators: Calcium and zinc act as cofactors (3–6).

Usage Notes:

1. Resuspend Thermolysin in thermolysin digestion buffer (50mM Tris [pH 8.0], 0.5mM CaCl₂). Enzyme is soluble up to 1mg/ml in thermolysin digestion buffer. Store reconstituted Thermolysin at –20°C for up to 2 weeks.
2. The optimal digestion temperature range is 65–85°C.

Part# 9PIV400

Revised 1/18



AF9PIV400 0118V400



Promega

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Usage Information on Back

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Part# 9PIV400
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Signed by:

R. Wheeler, Quality Assurance

1. In-Solution Digestion Protocol

1. Resuspend the protein in thermolysin digestion buffer.
2. Resuspend Thermolysin in thermolysin digestion buffer.
3. Add Thermolysin to protein solution; mix. We recommend using enzyme:protein ratios of 1:20 to 1:50.
4. Incubate 30 minutes to 6 hours at 70–95°C. Lower temperatures will require longer incubation times.
5. Stop the reaction by adding 10% formic acid to a final concentration of 0.5%.

2. Composition of Buffers and Solutions

thermolysin digestion buffer

50mM	Tris-HCl (pH 8.0)
0.5mM	CaCl ₂

3. References

1. *Handbook of Proteolytic Enzymes* (1998) 1037–42.
2. Coffey, A. *et al.* (2000) *M. Appl. Microbiol.* **88**, 132–41.
3. Masahiro, T. *et al.* (1976) *Eur. J. Biochem.* **64**, 243–7.
4. Latt, S.A. *et al.* (1969) *Biochem. Biophys. Res. Comm.* **37**, 333.
5. Feder, J. *et al.* (1971) *Biochemistry* **10**, 4552–6.
6. Tsuru, D. *et al.* (1966) *Agric. Biol. Chem.* **30**, 856–82.

4. 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
Endoproteinase Lys-C, Sequencing Grade	5µg		V1071
Fetuin	500µg	10mg/ml	V4961
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
Protein Deglycosylation Mix	20 reactions		V4931
rLys-C, Mass Spec Grade	15µg		V1671
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073