

Certificate of Analysis

Chymotrypsin, Sequencing Grade

Part No.	Size
V106A	25µg

Description: Chymotrypsin, Sequencing Grade, is a serine endoproteinase that specifically cleaves peptide bonds at the C-termini of Tyr, Phe, Trp and Leu. Met, Ala, Asp and Glu may be cleaved at a much lower rate.

Biological Source: Bovine pancreas.

Molecular Weight: 25kDa.

Form: Lyophilized.

Specific Activity: At least 70 units/mg by BTEE (N-benzoyl-L-tyrosine ethyl ester) assay.

Unit Definition: One unit is defined as the amount of Chymotrypsin that will hydrolyze 1.0µmol of BTEE per minute at pH 7.8 and 25°C.

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

Usage Notes: Resuspend in 1mM HCl. We recommend resuspending in 25–50µl of HCl for a final concentration of 0.5–1µg/µl. Resuspended Chymotrypsin, Sequencing Grade, can be stored for up to one week at 4°C.

Part# 9PIV106

Printed 3/09



AF9PI V106 0309V106

Quality Control Assays

This lot passes the following Quality Control specifications:

Specific Activity: At least 70 units/mg of protein by BTEE assay.

Purity: Greater than or equal to 90% pure by SDS-PAGE analysis.



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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Signed by:

J. Stevens, Quality Assurance

Part# 9PIV106
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I. Protocol

Preparation of Protein

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

- Solubilization/Denaturation:** Dissolve protein in 100mM Tris-HCl, 10mM CaCl₂ (pH 8.0). Proteins that are difficult to dissolve or require denaturation for efficient digestion can be solubilized in a minimum volume in a denaturant such as 6–8M urea or 6M guanidine HCl at room temperature to 37°C for up to one hour. For some proteins, it may be beneficial to heat the sample to 60°C over this time period (95°C for 15–20 minutes for extreme cases). ProteaseMAX™ Surfactant can be used (0.01–0.2%) in 100mM Tris-HCl, 10mM CaCl₂ (pH 8.0) in a minimum volume and does not require heating to be effective.
- Disulphide Reduction:** To the dissolved protein add DTT (or β-mercaptoethanol) to a final concentration of 5mM; heat this sample at 50–60°C for 20 minutes.
- Alkylation:** Allow the reduced protein mixture to cool to room temperature, and add iodoacetamide to a final concentration of 15mM. Incubate in the dark for 15 minutes at room temperature.
- Finally adjust the reaction volume with 100mM Tris-HCl, 10mM CaCl₂ (pH 8.0) such that the urea or guanidine concentration is 1M or less or the ProteaseMAX™ Surfactant concentration is at or below 0.025%.

Enzyme Reconstitution

Dissolve lyophilized Chymotrypsin, Sequencing Grade, in 1mM HCl. We recommend using 25–50µl per digestion to obtain a final concentration 0.5–1µg/µl.

Digestion

Add Chymotrypsin, Sequencing Grade, to a final protease:protein ratio of 1:200 to 1:20 (w/w), and incubate sample for 2–18 hours at 25°C. The reaction may be stopped, if desired, by adding 0.5% trifluoroacetic acid.

Note: The presence of up to 1M guanidine or urea in the digestion may reduce the activity of Chymotrypsin, Sequencing Grade, by up to 20%. The addition of ProteaseMAX™ Surfactant up to the recommended amount will not reduce the activity of Chymotrypsin, Sequencing Grade.

II. Related Products

Product	Size	Cat. #
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg	V2071
	5 × 1mg	V2072
Trypsin Gold, Mass Spectrometry Grade	100µg	V5280
Sequencing Grade Modified Trypsin	100µg	V5111
Sequencing Grade Modified Trypsin, Frozen	100µg	V5113
Endoproteinase Lys-C, Sequencing Grade	5µg	V1071