Certificate of Analysis

Sequencing Grade Modified Trypsin:

Part No.	Name	Size
V511A	Sequencing Grade Modified Trypsin	20µg
V511B	Sequencing Grade Modified Trypsin	100µg

Cat.# V5111 contains:

Part No.	Name	Size
V511A	Sequencing Grade Modified Trypsin	5 × 20µg
V542A	Trypsin Resuspension Buffer	1ml

Cat.# V5117 contains:

Part No.	Name	Size
V511B	Sequencing Grade Modified Trypsin	100µg
V542A	Trypsin Resuspension Buffer	1ml

Description: Trypsin specifically hydrolyzes peptide bonds at the carboxyl side of lysine and arginine residues. Unmodified trypsin is subject to auto-proteolysis, generating fragments that can interfere with protein sequencing or HPLC peptide analysis. In addition, auto-proteolysis can result in the generation of pseudotrypsin, which has been shown to exhibit chymotrypsin-like specificity (1). Promega Sequencing Grade Modified Trypsin is porcine trypsin modified by reductive methylation, rendering it resistant to proteolytic digestion (2). In enzymatic stability tests, modified trypsin was found to retain greater than two times the activity of unmodified trypsin.

Sequencing Grade Modified Trypsin is further improved by TPCK treatment followed by affinity purification, yielding a highly active and stable molecule. Sequencing Grade Modified Trypsin is provided in 20µg aliquots with a stability-optimized resuspension buffer. A protease:protein ratio of 1:100 to 1:20 (w/w) is recommended for protein sequencing.

Physical Form: Sequencing Grade Modified Trypsin is supplied lyophilized.

Resuspension Buffer (supplied with V5111 and V5117): Trypsin Resuspension Buffer (V542A) is composed of 50mM acetic acid.

Specific Activity: See the Product Information Label.

Storage Conditions: Store the lyophilized powder at –20°C. Store reconstituted enzyme at –70°C. See the Product Information Label for the expiration date.

Unit Definition: One unit is the amount of Sequencing Grade Modified Trypsin required to produce a ΔA_{253} of 0.001 per minute at 30°C with the substrate N_{α}-benzoyl-L-arginine ethyl ester (BAEE). The substrate is dissolved in 50mM Tris-HCl, 1mM $CaCl_2$ (pH 7.6), and the enzyme is diluted in 50mM acetic acid.

Usage Notes:

1. For maximum activity, resuspend Sequencing Grade Modified Trypsin in the Trypsin Resuspension Buffer provided, and heat at 30°C for 15 minutes before use.
2. Specific activities may vary widely between suppliers. Procedures written for use of trypsin by weight may need to be optimized based on enzyme activity.
3. Thaw the reconstituted trypsin at room temperature, placing on ice immediately after thawing. Remove the amount of trypsin needed, then refreeze the unused portion. To maintain maximum product activity, limit the number of freeze-thaw cycles to five or dispense into single-use aliquots after resuspending.

Quality Control Assays

Stability: A 0.1mg/ml solution of Sequencing Grade Modified Trypsin retains at least 85% of its activity after a 3-hour incubation at 37°C in 40mM NH_4HCO_3.

Sequence Specificity: Fifty micrograms of insulin β-chain are incubated with 2.5µg of Sequencing Grade Modified Trypsin for 2 hours and for approximately 18 hours at 37°C. The digestion products are separated by reverse phase HPLC and detected at 215nm. The 18-hour digest shows the two main digestion products with no significant new peaks compared with the 2-hour digest.

Usage Information on Back

Signed by:

C. Stollberg, Quality Assurance

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Usage Information

1. Product Information

A. Specificity

Trypsin is a serine protease that specifically cleaves at the carboxylic side of lysine and arginine. Restrictions to the specificity of trypsin occur when proline is at the carboxylic side of lysine or arginine; the bond is almost completely resistant to cleavage by trypsin. Cleavage may also be considerably reduced when acidic residues are present on either side of a potentially susceptible bond (3).

B. Stability

Modified trypsin is maximally active in the pH range of 7–9 and reversibly inactivated at pH 4. It is resistant to mild denaturing conditions: 0.1% SDS, 1M urea, or 10% acetonitrile (4). Modified trypsin retains 48% activity in 2M guanidine HCl (3).

2. Protocol

A. Protein Denaturation

In general, proteins require denaturation and disulfide bond cleavage before enzymatic digestion can go to completion (3).

Dissolve 1–10mg of the target protein in 6M guanidine HCl (or 6–8M urea), 50mM Tris-HCl (pH 8), 2–4mM DTT (or β-mercaptoethanol) in a reaction volume of up to 1ml (25µl minimum). Heat at 95°C for 15–20 minutes or at least 60°C for 45–60 minutes. If smaller amounts of protein are to be digested, the recommended conditions given can be scaled down proportionally. However, under no conditions should less than 25µl of dissolving agent be used.

After denaturation, allow the reaction to cool and add 50mM NH_4HCO_3 (pH 7.8) or 50mM Tris-HCl, 1mM $CaCl_2$ (pH 7.6), until the guanidine-HCl or urea concentration is below 1M.

B. Protease Digestion

Add modified trypsin to a final protease:protein ratio of 1:100 to 1:20 (w/w). Incubate at 37°C for at least 1 hour. Remove a small aliquot and chill the reaction on ice or freeze. Add an inhibitor to the aliquot to terminate the protease activity or precipitate the sample by the addition of TCA to a 10% final concentration. Determine the extent of digestion by subjecting a portion of the digestion products to reverse phase HPLC or SDS-PAGE. If further proteolysis is required, return the reaction tube to 37°C and continue incubating until the desired digestion is obtained (5). The reaction can be terminated by freezing or by the addition of specific inhibitors. Trypsin can also be inactivated by lowering the pH of the reaction to below 4. Trypsin will regain activity as the pH is raised above 4 (3). Reducing the temperature will decrease the digestion rate. Longer incubations, up to 24 hours, may be required depending on the nature of the protein. If using long incubations, be very careful to avoid bacterial contamination.

If a partial digestion of a nondenatured substrate is desired, as would be necessary for analysis of the domain structure of a protein, incubate the protein with modified trypsin at a protease:protein ratio of 1:100 to 1:20 in a buffer compatible with the stability of the target protein.

3. References

1. Keil-Dlouha, V. *et al.* (1971) Proteolytic activity of pseudotrypsin. *FEBS Lett.* **16**, 291–95.
2. Rice R.H. *et al.* (1977) Stabilization of bovine trypsin by reductive methylation. *Biochem. Biophys. Acta* **492**, 316–21.
3. Wilkinson, J.M. (1986) "Fragmentation of Polypeptides by Enzymic Methods". In: *Practical Protein Chemistry: A Handbook.* A. Darbre, ed., John Wiley and Sons, New York, N.Y.
4. Bond, J.S. (1989) "Commercially Available Proteases", Appendix II. In: *Proteolytic Enzymes, A Practical Approach.* R.J. Beynon and J.S. Bond, eds., IRL Press, Oxford, U.K.
5. Flannery, A.V., Beynon, R.J. and Bond, J.S. (1989) "Proteolysis of Proteins for Sequencing Analysis and Peptide Mapping". In: *Proteolytic Enzymes: A Practical Approach.* R.J. Beynon and J.S. Bond, eds., IRL Press, Oxford, U.K.

4. Related Products

Product	Size	Cat.#
Asp-N, Sequencing Grade	2µg	V1621
Chymotrypsin, Sequencing Grade	25µg	V1061
	100µg (4 × 25µg)	V1062
Elastase	5mg	V1891
Endo H	10,000u	V4871
	50,000u	V4875
Fetuin	500µg	V4961
Glu-C, Sequencing Grade	50µg (5 × 10µg)	V1651
Immobilized Trypsin	2ml	V9012
	4ml (2 × 2ml)	V9013
Pepsin	250mg	V1959
PNGase F	500u	V4831
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg	V2071
	5 × 1mg	V2072
rLys-C, Mass Spec Grade	15µg	V1671
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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