Certificate of Analysis

Sequencing Grade Modified Trypsin, Frozen:

Part# 9PIV5113

Revised 4/18

Part No.	Name	Size
V5113	Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)
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's Sequencing Grade Modified Trypsin, Frozen, 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, Frozen, is further improved by TPCK treatment followed by affinity purification, yielding a highly active and stable molecule. Sequencing Grade Modified Trypsin, Frozen, 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, Frozen, is supplied as a frozen liquid in 50mM acetic acid.

Protein Concentration: 0.5 ± 0.05mg/ml (determined by measuring A_{280}).

Resuspension Buffer (supplied): Trypsin Resuspension Buffer (V542A) is composed of 50mM acetic acid. This buffer may be used to further dilute the trypsin.

Specific Activity: See the Product Information Label.

Storage Conditions: Store at –70°C. See the Product Information Label for the expiration date.

Unit Definition: One unit is the amount of Sequencing Grade Modified Trypsin, Frozen, 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. Specific activities may vary widely between suppliers. Procedures written for use of trypsin by weight may need to be optimized based on enzyme activity.
2. To use this product, thaw at 25–30°C, mix gently and keep on ice until use. Do not keep thawed trypsin on ice for more than 1 hour. If the content of one vial is not used in its entirety, dispense the remaining trypsin into single-use aliquots and store at –70°C.
3. 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.

Signed by:

R. Wheeler, 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 is 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 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. 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
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

4. References

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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.
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