

## Certificate of Analysis

### Sequencing Grade Modified Trypsin, Frozen:

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<sub>280</sub>).

**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<sub>253</sub> of 0.001 per minute at 30°C with the substrate N<sub>α</sub>-benzoyl-L-arginine ethyl ester (BAEE). The substrate is dissolved in 50mM Tris-HCl, 1mM CaCl<sub>2</sub> (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<sub>4</sub>HCO<sub>3</sub>.

**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.

Part# 9PIV5113

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Signed by:

J. Stevens, Quality Assurance

## I. 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).

## II. 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  $\beta$ -mercaptoethanol) in a reaction volume of up to 1ml (25 $\mu$ l 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 25 $\mu$ l of dissolving agent be used.

After denaturation, allow the reaction to cool and add 50mM  $\text{NH}_4\text{HCO}_3$  (pH 7.8) or 50mM Tris-HCl, 1mM  $\text{CaCl}_2$  (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.

## III. Related Products

Product	Size	Cat.#
Proteinase K*	100mg	V3021
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg	V2071
Trypsin Gold, Mass Spectrometry Grade	100 $\mu$ g	V5280
Endoproteinase Lys-C, Sequencing Grade	5 $\mu$ g	V1071
Chymotrypsin, Sequencing Grade	25 $\mu$ g	V1061
	4 × 25 $\mu$ g	V1062

\*For Laboratory Use.

## IV. References

- Keil-Dlouha, V. (1971) Proteolytic activity of pseudotrypsin. *FEBS Lett.* **16**, 291–95.
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- 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.
- 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. **240**.
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