

## Modified Trypsin (TPCK-treated)



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www.neb.com



P8101S 006120613061

# P8101S

**100 µg**      **Lot: 0061206**      **Exp: 6/13**  
**5 x 20 µg**      **Store at -20°C**

**Description:** Modified Trypsin (TPCK-treated) is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues (1). Modified Trypsin is treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inactivate any remaining chymotryptic activity. It is modified by acetylation of the ε-amino groups of lysine residues to prevent autolysis. Modified Trypsin

**Now 2X Reaction Buffer**

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(TPCK-treated) cleaves at Lys-Pro and Arg-Pro bonds at a much slower rate than other amino acid residues (2).

**Source:** Isolated from bovine (*Bos taurus*) pancreas

### Applications:

- Digestion of proteins for proteomic analysis by Mass Spectrometry
- Protein and peptide identification

**Reaction Conditions:** 1X Modified Trypsin Reaction Buffer. Incubate at 37°C.

**Reagents Supplied with Enzyme:**  
2X Modified Trypsin Reaction Buffer.

### 1X Modified Trypsin Reaction Buffer:

50 mM Tris-HCl  
20 mM CaCl<sub>2</sub>  
pH 8.0 @ 25°C

**Note:** Substrate must be in phosphate-free buffer to prevent calcium precipitation with both reconstituted enzyme and enzyme buffer.

**Specific Activity:** 2.1 µmol/min/mg

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**Specific Activity:** 2.1 µmol/min/mg

**Molecular Weight:** 23,675 daltons

**Reconstitution:** Modified Trypsin should be reconstituted by the addition of 20–200 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

**Storage Conditions:** Supplied freeze-dried from a sodium acetate and calcium chloride buffer. Store at -20°C.

Can be stored frozen in solution at -20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

**Quality Assurance:** Modified Trypsin (TPCK-treated) is free of glycerol and detergents which may interfere with Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) Mass Spectrometry (MS) or liquid chromatography (LC) methods.

### Quality Controls

#### Modified Trypsin Activity:

Measured in three assays:

1. Protein digestion and analysis by MALDI-TOF MS

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#### Modified Trypsin Activity:

Measured in three assays:

1. Protein digestion and analysis by MALDI-TOF MS

2. Peptide digestion and analysis by MALDI-TOF MS
3. Fluorometric substrate digestion and specific activity determination in 1X Modified Trypsin Reaction Buffer.

### Protein Digestion: *Issatchenkia orientalis*

Cytochrome c (Swiss-Prot: CYC\_ISSOR) (Sigma) is subjected to digestion by Modified Trypsin at a ratio of 20:1 respectively for 16 hours at 37°C in Modified Trypsin Reaction Buffer. 1 µl of the above reaction (50 ng) was mixed with 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution, air-dried and subjected to MALDI-TOF MS analysis.

**Peptide Digestion:** A modified Human calcitonin peptide is subjected to digestion by Modified Trypsin at a ratio of 20:1 respectively for 16 hours at 37°C in Modified Trypsin Reaction Buffer. 1 µl of the above reaction (10 ng) was mixed with 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution, air-dried and subjected to MALDI-TOF MS analysis

(see other side)

CERTIFICATE OF ANALYSIS

2. Peptide digestion and analysis by MALDI-TOF MS
3. Fluorometric substrate digestion and specific activity determination in 1X Modified Trypsin Reaction Buffer.

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### Modified Trypsin Protein Sequence:

1 IVGGYTCAENSVPYQVSLNAGYHFCGGSLINDQWVVSAAHCYQYHIQVRLGEYNID  
61 VLEGGEQFIDASKIIRHPKYSSWTLNDNDILLIKLSTPAVINARVSTLLLPSACASA  
121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG  
181 GKDSCQGDSSGGPVACNGQLQGI VSWGYGCAQK GKPGVYTKVCNYVDWIQETIAANS

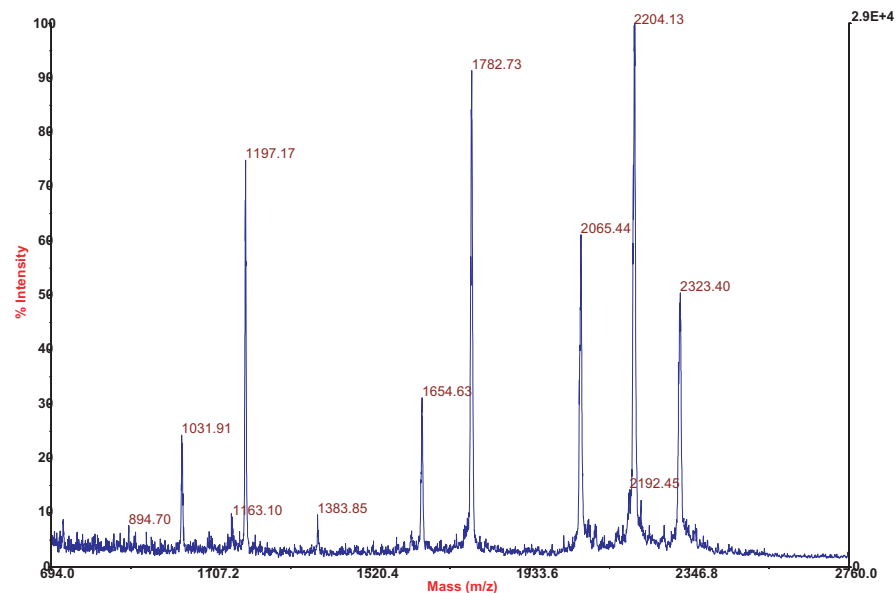
**Fluorometric Assay:** 250 ng (~1  $\mu$ mol) of Ala-Phe-Lys 7-amidomethyl coumarin peptide was suspended in 150  $\mu$ l of Modified Trypsin Reaction Buffer and 1  $\mu$ g of Modified Trypsin was added. The initial rate was determined by measurement of the increase in fluorescence (excitation 365 nm and emission 440 nm). The protein concentration is determined by C18 reverse-phase LC and integration.

**Note:** Modified Trypsin is acetylated on multiple lysine residues. This protein appears as a single band on SDS-PAGE. This sequence is also available at [www.neb.com](http://www.neb.com).

### References:

1. Northrop J. H. and Kunitz, M. (1931). *Science* 73, 262–263.
2. Perona J. J. and Craik, C.S. (1995). *Protein Sci.* 4, 337–360.

**MALDI-TOF MS:** *Issatchenka orientalis* Cytochrome c subjected to digestion by Modified-Trypsin for 16 hours, dried and subjected to MALDI-TOF MS.



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121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG  
181 GKDSCQGDSSGGPVACNGQLQGI VSWGYGCAQK GKPGVYTKVCNYVDWIQETIAANS

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