Modified Trypsin (TPCK-treated)

1-800-632-7799
info@neb.com
www.neb.com

P8101S 006120813081

P8101S

100 µg **Lot: 0061208** **Exp: 8/13**

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

Now 2X Reaction Buffer

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 $CaCl_2$
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

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
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

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(see other side)

CERTIFICATE OF ANALYSIS

Modified Trypsin Protein Sequence:

```
  1 IVGGYTCAENSVPYQVSLNAGYHFCGGSLINDQWVVSAAHCYQYHIQVRLGEYNID
 61 VLEGGEQFIDASKIIRHPKYSSWTLDNDILLIKLSTPAVINARVSTLLLPSACASA
121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG
181 GKDSCQGDSGGPVACNGQLQGIVSWGYGCAQKGKPGVYTKVCNYVDWIQETIAANS
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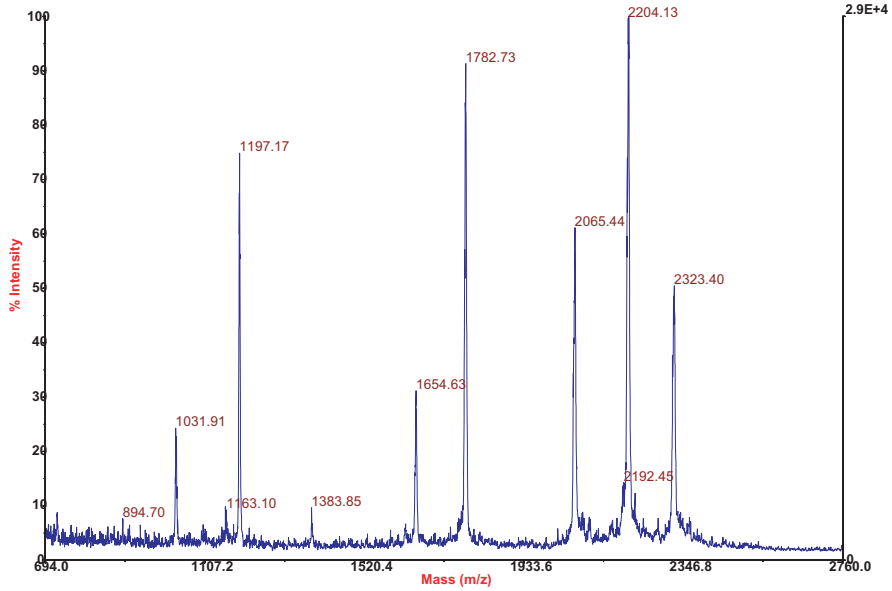
Fluorometric Assay: 250 ng (~1 µmol) of Ala-Phe-Lys 7-amidomethyl coumarin peptide was suspended in 150 µl of Modified Trypsin Reaction Buffer and 1 µg of Modified Trypsin was added. The initial rate was determined by measurement of the increase in fluorescence (excitation 365 nm and emission 440 nm). 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.

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



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121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG
181 GKDSCQGDSGGPVACNGQLQGIVSWGYGCAQKGKPGVYTKVCNYVDWIQETIAANS
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