Trypsin-ultra™, Mass Spectrometry Grade

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

P8101S 006130114011

P8101S

100 µg **Lot: 0061301** **Exp: 1/14**

5 x 20 µg **Store at –20°C**

Description: Trypsin-ultra, Mass Spectrometry Grade is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues (1). Trypsin-ultra 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. Trypsin-ultra (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 Trypsin-ultra, Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X Trypsin-ultra, Reaction Buffer.

1X Trypsin-ultra, 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: Trypsin-ultra, Mass Spectrometry Grade 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: Trypsin-ultra, Mass Spectrometry Grade 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

Trypsin-ultra, Mass Spectrometry Grade 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 Trypsin-ultra, Mass Spectrometry Grade Reaction Buffer.

Protein Digestion: *Issatchenkia orientalis* Cytochrome c (Swiss-Prot: CYC_ISSOR) (Sigma) is subjected to digestion by Trypsin-ultra, Mass Spectrometry Grade at a ratio of 20:1 respectively for 16 hours at 37°C in Trypsin-ultra, Mass Spectrometry Grade 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 Trypsin-ultra, Mass Spectrometry Grade at a ratio of 20:1 respectively for 16 hours at 37°C in Trypsin-ultra, Mass Spectrometry Grade 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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CERTIFICATE OF ANALYSIS

Trypsin-ultra, Mass Spectrometry Grade Protein Sequence:

```
  1 IVGGYTCAENSVPYQVSLNAGYHFCGGSLINDQWVVSAAHCYQYHIQVRLGEYNID
 61 VLEGGEQFIDASKIIRHPKYSSWTLDNDILLIKLSTPAVINARVSTLLLPSACASA
121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG
181 GKDSCQGDSGGPVACNGQLQGIVSWGYGCAQKGKPGVYTKVCNYVDWIQETIAANS
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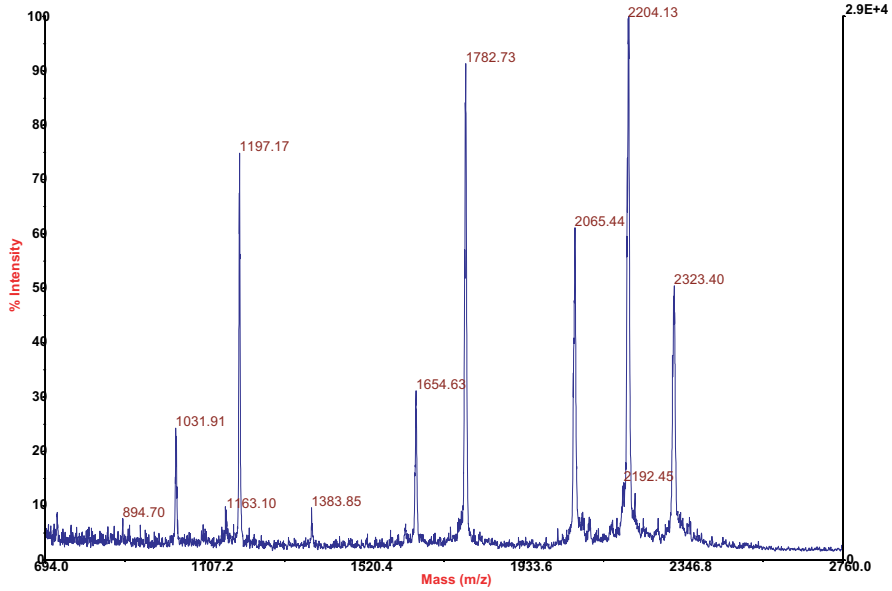
Fluorometric Assay: 250 ng (~1 µmol) of Ala-Phe-Lys 7-amidomethyl coumarin peptide was suspended in 150 µl of Trypsin-ultra, Mass Spectrometry Grade Reaction Buffer and 1 µg of Trypsin-ultra, Mass Spectrometry Grade was added. The initial rate was determined by measurement of the increase in fluorescence (excitation 365 nm and emission 440 nm). The protein concentration is determined by C18 reverse-phase LC and integration.

Note: Trypsin-ultra, Mass Spectrometry Grade 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 Trypsin-ultra, Mass Spectrometry Grade for 16 hours, dried and subjected to MALDI-TOF MS.



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 61 VLEGGEQFIDASKIIRHPKYSSWTLDNDILLIKLSTPAVINARVSTLLLPSACASA
121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG
181 GKDSCQGDSGGPVACNGQLQGIVSWGYGCAQKGKPGVYTKVCNYVDWIQETIAANS
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