Modified Trypsin (TPCK-treated)

**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 is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues (2). 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.

**Application:**
- 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.

**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:**
- Measured in three assays:
  1. Protein digestion and analysis by MALDI-TOF MS

**Molecular Weight:** 23,675 daltons

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

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

**Quality Controls**
- Modified Trypsin Activity: Measured in three assays:
  1. Protein digestion and analysis by MALDI-TOF MS
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:

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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