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PTMScan® Trypsin, TPCK-Treated



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20 mg

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New 01/18

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Description: Trypsin is a serine endopeptidase derived from its inactive pancreatic zymogen, trypsinogen, when the N-terminal 6 amino acid leader sequence is enzymatically removed. Activated trypsin cleaves amide and ester bonds of lysine and arginine and is used extensively in detaching cells from culture dishes, protein sequencing, and proteomics applications.

Background: Trypsin digests polypeptides by hydrolysis at the carboxyl side of unmodified arginine and lysine residues (1). Proteolysis is slower when the cleavage site is flanked by acidic residues and will not occur if the lysine or arginine is followed by a proline (2-5). Trypsin's activity is optimal at pH 8.0 and is inhibited to varying degrees by organophosphorus compounds such as diisopropyl fluorophosphate, as well as EDTA, aprotinin, Ag+, and benzamidine (6-8).

Background References:

- (1) Brown, W.E. and Wold, F. (1973) Biochemistry 12, 828-34.
- (2) Rodriguez, J. et al. (2008) J Proteome Res 7, 300-5.
- (3) Leiros, H.K. et al. (2004) Protein Sci 13, 1056-70.
- (4) Rawlings, N.D. and Barrett, A.J. (1994) Methods Enzymol 244, 19-61.
- (5) Perona, J.J. and Craik, C.S. (1995) Protein Sci 4, 337-60.
- (6) Kostka, V. and Carpenter, F.H. (1964) *J Biol Chem* 239, 1799-803.
- (7) Levilliers, N. et al. (1970) Arch Biochem Biophys 140, 474-83.
- (8) Polgár, L. (2005) Cell Mol Life Sci 62, 2161-72.

Specificity/Sensitivity: One unit of trypsin hydrolyzes 1 µmole of p-toluene-sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C, pH 8.0 in the presence of 10 mM calcium. One mg of trypsin is approximately equivalent to 180 TAME units.

Source/Purification: Trypsin is chromatographically purified from bovine pancreas, treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit chymotryptic activity, 0.22 micron diafiltered against 1 mM HCl, and lyophilized.

Directions for Use: PTMScan® Trypsin-TPCK is provided for use with Cell Signaling Technology's patented PTMScan® protocol in the initial protein digestion step. Samples in urea lysis buffer need to be diluted to 2M or less urea prior to addition of trypsin. For a 1 mg/mL solution, dissolve 20 mg trypsin in 4 mL of 1 mM HCl prior to use. Transfer the 4 mL to a 50 mL conical tube and bring the volume up to 20 mL with 1 mM HCl. Aliquot and store at -80°C. Consult the specific PTMScan® kit and protocol for more details on the appropriate protease before digesting any protein samples.

Storage: Store lyophilized trypsin powder at 4°C protected from moisture. Once reconstituted, store trypsin solutions at -80°C. Lyophilized trypsin has a shelf life of 1 year at 4° C and solutions are stable for 6 months at -80° C.

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