Trypsin from *Gadus morhua* (Atlantic cod)

Product Number  T 9906

**Product Description**

Enzyme Commission (EC) Number: 3.4.21.4  
Molecular Weight: 24.2 kDa  
pl: 5.5-6.6  
Synonyms: Atlantic cod trypsin

Trypsin consists of a single chain polypeptide of 223 amino acid residues. Trypsin is produced by the removal of the N-terminal hexapeptide from trypsinogen which is cleaved at the Lys<sup>6</sup>-Ile<sup>7</sup> peptide bond. The amino acid sequence of trypsin is cross-linked by 6 disulfide bridges. This native form of trypsin is referred to as β-trypsin. Autolysis of β-trypsin (which is cleaved at Lys<sup>131</sup>-Ser<sup>132</sup>) results in α-trypsin which is held together by disulfide bridges. Trypsin is a member of the serine protease family. The active site amino acid residues of trypsin include His<sup>46</sup> and Ser<sup>183</sup>.<sup>2,3</sup>

Trypsin will cleave peptides on the C-terminal side of lysine and arginine amino acid residues. The rate of hydrolysis is slower if an acidic residue is on either side of the cleavage site and no cleavage occurs if a proline residue is on the carboxyl side of the cleavage site. The pH optimum of trypsin from Atlantic cod is 8.0.<sup>1</sup> Trypsin will also hydrolyze ester and amide linkages of synthetic derivatives of amino acids such as: benzoyl-L-arginine ethyl ester (BAEE), p-toluenesulfonyl-L-arginine methyl ester (TAME), tosyl-L-arginine methyl ester, N<sub>α</sub>-benzoyl-L-arginine p-nitroanilide (BAPNA), L-lysyl-p-nitroanilide, and benzoyl-L-tyrosine ethyl ester (BTEE). Reported K<sub>m</sub> values for Atlantic cod trypsin include TAME (29 µM) and BAPNA (77 µM).<sup>1,2,3,4,5</sup>

The oxidized B chain of insulin is often used as a substrate to determine the suitability of trypsin for use in protein sequencing. The presence of two peptide bonds (Arg<sup>22</sup>-Gly<sup>23</sup> and Lys<sup>29</sup>-Ala<sup>30</sup>) make it an ideal peptide for use in this kind of application.<sup>6</sup>

Serine protease inhibitors that inhibit Atlantic cod trypsin include TLCK (N<sub>α</sub>-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), benzamidine, soybean trypsin inhibitor, and ovomucoid.<sup>1</sup>

**Precautions and Disclaimer**

For Laboratory Use Only. Not for drug, household or other uses.

**Preparation Instructions**

This enzyme is soluble in 67 mM sodium phosphate buffer, pH 7.6 (5 mg/ml), yielding a clear solution.

**Storage/Stability**

Unlike bovine and porcine trypsin, Atlantic cod trypsin is unstable at low pH values (less than 5.0). In addition, calcium has not been found to stabilize solutions of trypsin and to prevent autolysis (self-digestion).<sup>1</sup>

**Procedure**

For trypsin digestion of peptides, use a ratio (w:w) of about 1:100 to 1:20 for trypsin:peptide. Trypsin preparations usually contain some contaminating chymotrypsin and this should be inhibited with N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK).<sup>7</sup>

**References**

