**Product Information**

**Trypsin TPCK treated from bovine pancreas aseptically filled**

**Catalog Number** T8802  
**Storage Temperature** –20 °C

- **CAS RN** 9002-07-7  
- **EC** 3.4.21.4
- **Molecular mass:** 1224 kDa  
- **Extinction Coefficient:** 3.4 E1% = 12.9–15.4 (280 nm)  
- **pI:** 2.5

**Synonyms:** Tryptase, Tryptar, Cocoonase, Parenzyme, Parenzymol

**Product Description**

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 Lys6–Ile7 peptide bond. The amino acid sequence of trypsin is crosslinked by 6 disulfide bridges. This native form of trypsin is referred to as β-trypsin. Autolysis of β-trypsin (which is cleaved at Lys131–Ser132 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 His46 and Ser183.

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 is 7–9. Trypsin will also hydrolyze ester and amide linkages of synthetic derivatives of amino acids such as: benzoyl L-arginine ethyl ester (BAEE), p-toluene sulfonyl L-arginine methyl ester (TAME), tosyl-L-arginine methyl ester, Nα-benzoyl-L-arginine p-nitroanilide (BAPNA), L-lysyl p-nitroanilide, and benzoyl-L-arginamide.

Reported K_M values are BAEE (0.05 mM), TAME (0.05 mM), and BAPNA (0.94 mM).

Assuming the pH and temperature are the same and using a molar extinction coefficient of 808 at 254 nm for BAEE, the following conversions are valid:

- 1 BAEE μM Unit = 200 BAEE A_253 Units
- 1 TAME μM Unit = 0.27 BAEE μM Units
- 1 BAEE μM Unit = 3.64 TAME Units
- 1 TAME μM Unit = 55 BAEE A_253 Units
- 1 BAEE A_253 Unit = 0.018 TAME μM Unit
- 1 TAME μM Unit = 180 TAME A_247 Units
- 1 TAME A_247 Unit = 0.33 BAEE Units
- 1 USP Unit = ΔA_253 of 0.003 per minute
- 1 NF Unit = 3.3 A_253 BAEE Units.

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 (Arg22–Gly23 and Lys29–Ala30) make it an ideal peptide for use in this kind of application.

Serine protease inhibitors that will inhibit trypsin include DFP (diisopropyl fluorophosphate), TLCK (Nα-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), APMSF (4-amidinophenylethyl-sulfonl fluoride), AEBSEF (4-(2-aminoethyl)benzenesulfonyl fluoride), aprotinin, leupeptin, α2-macroglobulin, α1-antitrypsin, p-aminobenzamidine, benzamidine (reversible), soybean trypsin inhibitor, lima bean inhibitor, bovine pancreas trypsin inhibitor, chicken egg white inhibitor, and turkey egg white inhibitor.

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

This enzyme is soluble in 1 mM HCl (1 mg/ml), yielding a clear solution.
Storage/Stability
Solutions in 1 mM HCl (pH 3) remain active for ~1 year when aliquoted and stored at -20 °C. The presence of calcium (20 mM) will also retard the autolysis of trypsin and maintain the stability of trypsin in solution.\(^2,6\) Trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% (w/v) SDS.\(^3\) Trypsin is reversibly denatured at high pH (above 11), by precipitation with TCA, or by high concentrations of urea (greater than 6.5 M).\(^3\) In order to abolish all trypsin activity, heating at 100 °C in 1% (w/v) SDS for 5 minutes is required.\(^4\)

Procedure
For trypsin digestion of peptides, use a ratio (w/w) of 1:100 to 1:20 for trypsin:peptide. Trypsin preparations usually contain some contaminating chymotrypsin and should be inhibited with N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK).\(^12\) This product has been treated with TPCK to inhibit chymotrypsin activity.

References
4. Shaw, E. et al., Evidence for an active center histidine in trypsin through use of a specific reagent, 1-chloro-3-tosylamido-7-amino-2-heptanone, the chloromethyl ketone derived from N\(_\alpha\)-tosyl-L-lysine. Biochemistry, \(4\)(10), 2219-2224 (1965).