Trypsin from bovine pancreas
Suitable for protein sequencing

Catalog Number T8658
Storage Temperature −20 °C

CAS RN 9002-07-05
EC 3.4.21.4
Molecular mass: 24 kDa
Extinction Coefficient: E1%1cm = 12.9–15.4 (280 nm)
pl = 10.1–10.5
pH optimum: 6.7–9

Synonyms: Tryptase, Tryptar, Cocoonase, Parenzyme, Parenzymol

Product Description
Trypsin is a member of the serine protease family. The active site amino acid residues of trypsin include His46 and Ser183.2,4 Trypsin consists of a single chain polypeptide of 223 amino acid residues. Trypsin is produced by the cleavage of the N-terminal hexapeptide from its precursor, trypsinogen, at the Lys6–Ile7 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 by cleavage at its Lys131–Ser132 bond results in α-trypsin, which is held together by disulfide bridges.

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. 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, Nα-benzoyl-L-arginine p-nitroanilide (BAPNA), L-lysyl p-nitroanilide, and benzoyl-L-arginamide.2,7,8

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

Assuming that 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 Units
1 TAME µM Unit = 0.27 BAEE µM Units
1 BAEE µM Unit = 3.64 TAME Units
1 TAME µM Unit = 55 BAEE A253 Units
1 BAEE A253 Unit = 0.018 TAME µM Unit
1 TAME µM Unit = 180 TAME A247 Units
1 TAME A247 Unit = 0.33 BAEE Units
1 USP Unit = ΔA253 of 0.003 per minute
1 NF Unit = 3.3 A253 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) makes it an ideal peptide for use in this kind of application.10

Serine protease inhibitors that will inhibit trypsin include DFP (diisopropyl fluorophosphate), TLCK (Nα-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), APMSF (4-amidinophenylmethane-sulfonyl fluoride), AEBSF (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.2,11

Electrospray mass spectrometry has been used to study the molecular mass of bovine trypsin.12 The crystal structure of bovine trypsin has been reported.13

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.³,⁵

Trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% (w/v) SDS.¹⁴ 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.⁷⁵

Procedure
For trypsin digestion of proteins, use a ratio (w:w) of 1:100 to 1:20 for trypsin:protein. This product has been suitability-tested as a sequencing grade protease. This suitability is demonstrated by a prolonged digestion of oxidized insulin β chain (100 µg of oxidized β chain of insulin is digested with 5 µg of trypsin for 18 hours, at 37 °C, in 0.1 ml of Tris HCl buffer, pH 8.5). During the 18-hour digestion, only the expected peptides are generated with no indication of other proteolytic activity. Under these conditions, the cleavage of the test peptide is complete in less than 5 minutes.

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 Nα-tosyl-L-lysine. Biochemistry, 4(10), 2219-2224 (1965).