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Product Information

Trypsin from bovine pancreas
Type I

Catalog Number **T8003**
Storage Temperature –20 °C

CAS RN 9002-07-05
EC 3.4.21.4
Molecular mass:[1,2] 24 kDa
Extinction Coefficient:[3,4] $E^{1\%}$ = 12.9–15.4 (280 nm)
pI:[2,5] = 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 His^{46} and Ser^{183}.[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 Lys^{6}–Ile^{7} 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 Lys^{131}–Ser^{132} 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 K_M 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 Unit = 0.27 BAEE μM Unit Units
1 BAEE μM Unit Unit = 3.64 TAME Units
1 TAME μM Unit Unit = 55 BAEE A_{253} Units
1 BAEE A_{253} Unit = 0.018 TAME μM Unit Unit
1 TAME μM Unit Unit = 180 TAME A_{247} Units
1 TAME A_{247} Unit = 0.33 BAEE Units
A USP Unit = ΔA_{253} of 0.003 per minute
1 NF Unit = 3.3 A_{253} BAEE Units.[9]

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^{22}–Gly^{23} and Lys^{29}–Ala^{30}) 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 (N_α-*p*-tosyl-L-lysine chloromethyl ketone),
PMSF (phenylmethanesulfonyl fluoride),
APMSF (4-amidinophenylmethane-sulfonyl fluoride),
AEBSF (4-(2-aminoethyl)benzenesulfonyl fluoride),
aprotinin, leupeptin, α_2-macroglobulin, α_1-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.[2,6]

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.[15]

Procedure

For trypsin digestion of proteins, use a ratio (w:w) of 1:100 to 1:20 for trypsin:protein. Trypsin preparations usually contain some contaminating chymotrypsin.

References

1. Cunningham, L.W., Jr., Molecular Kinetic properties of crystalline diisopropyl phosphoryl trypsin. J. Biol. Chem., **211(1)**, 13-19 (1954).
2. Walsh, K.A., Trypsinogens and trypsins of various species. *Meth. Enzymol*., **19**, 41-63 (1970).
3. Keil, B., in *The Enzymes*, 3rd ed., Vol. III, Boyer, P.D., Academic Press (New York, NY: 1971), pp. 250-275.
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^{α}-tosyl-L-lysine. *Biochemistry*, **4(10)**, 2219-2224 (1965).
5. Buck, F.F. *et al*., On the mechanism of enzyme action. LXXII. Studies on trypsins from beef, sheep, and pig pancreas. *Arch. Biochem. Biophys*., **97**, 417-424 (1962).
6. Sipos, T., and Merkel, J.R., An effect of calcium ions on the activity, heat stability, and structure of trypsin. *Biochemistry*, **9(14)**, 2766-2775 (1970).
7. Burdon, R.H., *et al*., in *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 9, 2nd ed., Allen, G., ed., Elsevier/North (New York, NY: 1989), pp. 73-104.
8. *Enzyme Handbook*, Vol. II, Barman, T.E., Springer-Verlag (New York, NY: 1969), pp. 618-619.
9. USP, Vol. 23, p. 1611.
10. Wang, S.-S., and Carpenter, F.H., Kinetics of the tryptic hydrolysis of the oxidized B chain of bovine insulin. *Biochemistry*, **6(1)**, 215-224 (1967).
11. *Proteolytic Enzymes, A Practical Approach*, Beynon, R. J., ed., IRL Press (New York, NY: 1989), p. 240.
12. Ashton, D.S., *et al.*, On the analysis of bovine trypsin by electrospray-mass spectrometry. *Biochem. Biophys. Res. Comm.*, **199(2)**, 694-698 (1994).
13. Stroud, R.M., *et al.*, The Structure of Bovine Trypsin: Electron Density Maps of the Inhibited Enzyme at 5 Å and 2.7 Å Resolution. *J. Mol. Biol.*, **83(2)**, 185-208 (1974).
14. *Methods of Molecular Biology*, Vol. 3, Smith, B.J., Humana Press, (New Jersey, 1988), pp. 57-69.
15. Porter, W.H., and Preston, J.L., Retention of trypsin and chymotrypsin proteolytic activity in sodium dodecyl sulfate solutions. *Anal. Biochem*., **66(1)**, 69-77 (1975).

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