Product Information

Trypsin from bovine pancreas

TPCK Treated, essentially salt-free, lyophilized powder, ≥10,000 BAEE units/mg protein

T1426

Product Description

CAS Registry Number: 9002-07-7

Enzyme Commission (EC) Number: 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

Trypsin is a member of the serine protease family. Its active site amino acid residues include His^{46} and Ser^{183}.[2-4] Trypsin consists of a single polypeptide chain 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 cleaves 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 also hydrolyzes ester and amide linkages of synthetic derivatives of amino acids such as:[2,7,8]

- 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
- benzoyl-L-arginamide

Reported K_m values are:

- BAEE (0.05 mM)
- TAME (0.05 mM)
- 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 may be used:[9]

- 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 (Arg^{22}–Gly^{23} and Lys^{29}–Ala^{30}) makes the oxidized B chain of insulin an ideal peptide for use in this kind of application.[10]

Serine protease inhibitors that will inhibit trypsin include:[2,11]

- DFP (diisopropyl fluorophosphate)
- TLCK (Nα-*p*-tosyl-L-lysine chloromethyl ketone)
- PMSF (phenylmethanesulfonyl fluoride)
- APMSF (4-amidinophenylmethanesulfonyl 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
- Turkey egg white inhibitor

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]

Several theses[14-16] and dissertations[17-27] have cited use of product T1426 in their research protocols.

Precautions and Disclaimer

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.

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.[28] Trypsin is reversibly denatured at high pH (> 11), by precipitation with TCA, or by high concentrations of urea (> 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.[29]

Preparation Instructions

This enzyme is soluble in 1 mM HCl (1 mg/mL).

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. Thus, this product has been treated with N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK)[30] to inhibit any chymotrypsin activity which may be present.

References

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