SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Trypsin
from porcine pancreas

Product Number **T 7168**
Storage Temperature -0 °C

Product Description

Enzyme Commission Number (EC): 3.4.21.4
CAS Number: 9002-07-05
Molecular Weight: 23.4 kDa[1]
Extinction Coefficient: $E^{1\%}$ = 15.0 (280 nm)[2]
pI: 10.2 - 10.8[1,2]
Synonyms: Tryptase, Tryptar, Cocoonase, Parenzyme, Parenzymol
Trypsin 1mg with Buffer Salts

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^{6} - Ile^{7} 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^{131} - Ser^{132} 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^{46} and Ser^{183}.[1,3]

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.[2] 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.[2,3,4,5] 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 A_{253}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 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.[6]
Note: These activity conversions were determined using bovine trypsin; however, they are thought to be similar for porcine trypsin.

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}) make it an ideal peptide for use in this kind of application.[7]

Serine protease inhibitors that will inhibit trypsin include DFP (diisopropyl fluorophosphate), TLCK(Nα-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), APMSF (4-amidinophenylmethanesulfonyl fluoride), AEBSEF (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.[1,8]

Precautions and Disclaimer

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

Preparation Instructions

One tablet dissolved in 1 ml of deionized water, yields 1 mg/ml Trypsin, 4 mM $CaCl_2$ in 200 mM Tris, pH 7.7, at 25 °C.

Storage/Stability
Solutions in 1 mM HCl (pH 3) are stable for approximately 1 year when aliquoted and stored at –20 °C. The presence of Ca^{2+} (20 mM) will also retard trypsin's ability to selfdigest itself (autolysis) and will maintain the stability of the trypsin in solution.[1,10]

Trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% (w/v) SDS.[11] 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.[12]

Procedure
The treatment of tissues with trypsin can enhance their histochemical staining. Tissue antigens, carbohydrates, and other markers can be more readily stained by digesting tissue sections with trypsin. Even though many antigens may be detectable in tissue sections after routine fixation and processing, a more sensitive method for their detection may be required. Trypsin disentigrates the protein matrix uncovering tissue antigens and other markers for improved histochemical staining.

Dissolve one tablet in 1 ml of deionized water. Place trypsin solution on tissue section for digestion. Reactivity is dependent upon the temperature, concentration, and incubation time. Initial conditions recommended for tissue digestion include utilizing a 0.1% (w/v) solution and incubation at 37 °C for 15-30 minutes.

References

1. Walsh, K. A., Trypsinogens and trypsins of various species. Meth. Enzymol., **19**, 41-63 (1970)
2. 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).
3. Keil, B., in The Enzymes, 3rd ed., Vol. III, Boyer, P. D., Academic Press (New York, NY: 1971), pp. 250-275.
4. 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.
5. Enzyme Handbook, Vol. II, Barman, T. E., Springer-Verlag (New York, NY: 1969), pp. 618-619.
6. USP, Vol. 23, p. 1611.
7. 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).
8. Proteolytic Enzymes, A Practical Approach, Beynon, R. J., ed., IRL Press (New York, NY: 1989), p. 240.
9. Carpenter, F. H., Treatment of trypsin with TPCK. Methods Enzymol., **11**, 237 (1967).
10. 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).
11. Methods of Molecular Biology, Vol. 3, Smith, B. J., Humana Press, (New Jersey, 1988), pp. 57-69.
12. Porter, W. H., and Preston, J. L., Retention of trypsin and chymotrypsin proteolytic activity in sodium dodecyl sulfate solutions. Anal. Biochem., **66**, 69-77 (1975).

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