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TECHNICAL INFORMATION

Catalog Number: 100461, 100465, 100468, 100475, 100478, 152272
alpha-Chymotrypsin

CAS #: 9004-07-3

E.C. 3.4.21.1

Description: Chymotrypsin preferentially catalyzes the hydrolysis of peptide bonds involving L-isomers of tyrosine, phenylalanine, and tryptophan. It also readily acts upon amides and esters of susceptible amino acids.

In addition to bonds involving aromatic amino acids, chymotrypsin catalyzes at a high rate the hydrolysis of bonds of leucyl, methionyl, asparaginyl, and glutamyl residues. A study has been made by Berezin and Martinek[1] and Baumann et al.[2].

A pancreas extract contains equal amounts of two forms of the zymogen: Chymotrypsinogen A, with a molecular weight of 25,000 and and isoelectric point of 9.1, and Chymotrypsinogen B (E.C.3.4.4.6), with an isoelectric point of 5.2. Together, the zymogens represent 32% of the protein content of pancreatic extracts. Dependent upon the conditions, chymotrypsinogen A may be activated to a-, p-, d-, b-, or g-chymotrypsin. Desnuelle[3] has provided a review of the activation and properties of chymotrypsin. The covalent structure of chymotrypsinogen A is given by Meloun et al.[4]

Extinction coefficient = 20.4.

Inhibitors: The enzyme is inhibited by heavy metals, the natural trypsin inhibitors to various degrees[5], an inhibitor from potato[6], and organophosphorus compounds. Gel filtration of chymotrypsin removes autolysis products and other contaminants[7]. The specificity of a-chloroketone as a-chymotrypsin inhibitor has been studied by Kumar and Hein[8]. Erlanger et al.[9] report phenothiazine-N-carbonyl chloride to be specific for chymotrypsin inhibition. Also inhibited by AEBSF, a-1-antitrypsin, Aprotinin, DFP, PMSF, TPCK and a-2-Macroglobulin.

Storage of Solutions: The enzyme is stable for days in solution at pH 3.0 when stored refrigerated.

Assay

Method: The reaction velocity is determined according to Hummel[10] by measuring an increase in absorbance at 256 nm resulting from the hydrolysis of benzoyl-L-tyrosine ethyl ester. One unit hydrolyzes one micromole of benzoyl-L-tyrosine ethyl ester (BTEE) per minute at pH 7.8 and 25ºC under the specified conditions.

45 BTEE units = 10,000 optical density units = 1,330 N.F. (ATEE) units

Reagents

– 0.08 M Tris HCl buffer, pH 7.8 containing 0.1 M calcium chloride
– 0.00107 M Benzoyl-L-tyrosine ethyl ester (BTEE) in 50% w/w methanol (63 ml absolute methanol added to 50 ml reagent grade water)
– 0.001 N HCl

Enzyme

Dissolve enzyme at one mg/ml in 0.001 N HCl. Dilute in 0.001 N HCl to 10-30 ug/ml for assay.

mg protein/ml = A208/ml X 0.49

Procedure

Adjust the spectrophotometer to 256 nm and 25ºC.

Pipette into cuvettes as follows:

0.08 M Tris HCl buffer, pH 7.8 with 0.1 M $CaCl_2$	1.5 ml
0.00107 M BTEE	1.4 ml

Incubate in spectrophotometer at 25ºC for 4-5 minutes to achieve temperature equilibrium and record blank rate, if any. Add 0.1 ml of appropriately diluted enzyme and record increase in absorbance at 256 nm for 4-5 minutes. Calculate DA256/min from the initial linear portion of the curve.

Calculation

Units per mg = (DA256/min X 100) / (964* x mg/ml in the reaction mixture)

* Extinction coefficient of BTEE at 256 nm.

Availability:

Catalog Number	*Description*	*Size*
100478	a-Chymotrypsin from bovine pancreas, 3X crystallized, 40-50 units/mg protein	25 mg 100 mg
100461	a-Chymotrypsin from bovine pancreas, 3X crystallized, dialyzed, 40-50 units/mg protein	100 mg 250 mg 500 mg 1 gm
152272	a-Chymotrypsin from bovine pancreas, 1X crystallized, ≥35 units/mg protein	250 mg 500 mg 1 gm 5 gm
100468	b-Chymotrypsin from bovine pancreas, 20-30 units/mg protein	100 mg 1 gm
100475	g-Chymotrypsin from bovine pancreas, 2X crystallized, ~45 units/mg protein	25 mg 100 mg 1 gm
100465	d-Chymotrypsin from bovine pancreas, ~45 units/mg protein	100 mg 1 gm 5 gm

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