Thermolysin

T754920

Storage -20℃. Avoid freeze/thaw cycle. Store in the dark. Desiccated.

Introduction

Thermolysin is a heat-stable metalloprotease produced by Geobacillus stearothermophilus (Former: Bacillus stearothermophilus). Thermolysin specifically hydrolyzes peptide bonds on the amino side of bulky hydrophobic residues such as Leu, Ile, Val, and Phe. Thermolysin has one zinc ion necessary for activity and four calcium ions for stability. The amino acid sequence and three-dimensional structure of this enzyme have been determined.

Features

1) Amorphous powder, freeze-dried (Purity: Two times crystallized).
2) Optimum temperature: 65 - 70°C.
3) Optimum pH: 8.0.
4) Stable pH range: 5.0 - 8.5.
5) Stable temperature: Considerably stable at below 70°C.
6) Inhibitors: EDTA, SMPI (Streptomyces metalloprotease inhibitor).
7) Solubility: approx. 0.02% in dilute buffer solution; approx. 4% in 40-50% glycerin.
8) Molecular weight: 34,600.
9) E1% 1cm (280nm): 17.65 at pH 7.0.

The data for general enzyme features and enzyme activity are based only on our laboratory experiment results. Therefore, different experiment conditions may yield different results.

Specification

Protein digestive activity (pH 7.2): Not less than 7,000 PU/mg.

Assay method

This method is based on the Hagihara modification of the Anson method.

Unit definition

One protease unit (PU) is defined as the amount of the enzyme that will liberate non-proteinous digestion products from milk casein (final concentration 0.5%) to give a Folin's color equivalent to 1μg of tyrosine per minute at the early stage of the reaction at 35°C,

pH 7.2.The assay method is available on request.

Reagents

1) Substrate: 1% milk casein (according to Hammarsten) containing 1/15M Na_2HPO_4-KH_2PO_4 buffer (pH 7.55). The pH value of this solution should be 7.2.
2) TCA mixture: 0.1M trichloroacetic acid (TCA) containing 0.2M sodium acetate and 0.3M acetic acid.
3) 0.4M Na_2CO_3.
4) Diluted Folin's reagent: 5-fold.
5) Enzyme diluents: 0.01M borate buffer, pH 8.0, containing 0.002M $CaSO_4$ and 0.005% Triton X-100.
6) 42% Glycerin (pH 8.0): 42% glycerin adjusted to pH 8.0 with 0.01N NaOH, containing 0.005% Triton X-100.

Test preparation

Weigh accurately about 100mg of Thermolysin, dissolve it in 42% glycerin (pH 8.0), and bring to 100ml with 42% glycerin (pH 8.0). Dilute it with enzyme diluents so that 1ml of the final dilution will contain between 5 and 10 PU.

Procedure

1) Pipette 1.0ml of the test preparation into a test tube, and equilibrate it to 35°C.
2) Add 1.0ml of substrate pre-warmed to 35°C, and mix.
3) After exactly 10 minutes, add 2.0ml of TCA mixture to stop the reaction and incubate the mixture for a further 30 minutes at 35°C.
4) Filter the mixture with filter paper.
5) Pipette 1.0ml of the filtrate into a test tube, add 5.0ml of 0.4M Na_2CO_3 and then 1.0ml of diluted Folin's reagent, mix, and incubate the mixture for 20 minutes at 35°C.
6) Read the optical density at 660nm (OD_t).
7) Run a blank in which the order of adding substrate and TCA mixture is reversed (OD_b).

Calculation

Activity(PU/ml)=$(OD_t-OD_b)\times4/A\times10$

4: Total volume (ml)

10: Reaction time (min)

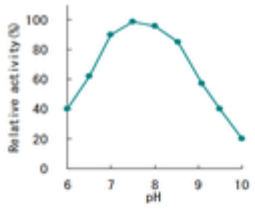
A: Optical density of 1μg/ml tyrosine solution (dissolved in 0.2N HCl) according to procedures 5 and 6

Handling caution

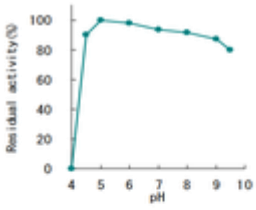
1) Do not inhale.
2) In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3) Please refer to MSDS for details.

General properties

The following results were obtained for the enzyme solution in some buffers. Please check appropriate conditions for your applications.

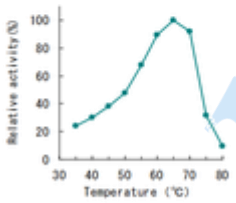


0.5% casein, Britton – Robinson buffer
35°C, 10min incubation



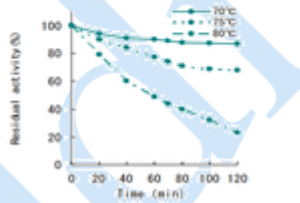
60°C, 30min incubation
buffer : pH4 – 6 acetic acid – sodium acetate
pH7 – 8 tris – acetic acid
pH9 – 12.5 glycine – sodium hydroxide

Temperature and Activity



0.5% casein, pH7.2, 10min incubation

Thermostability



0.01mmol/L tris-acetic acid buffer(2mmol/L $CaSO_4$, 5.26mmol/L NaCl), pH7.8