

# SERINE PROTEASE WITH CHYMOTRYPSIN SPECIFICITY FROM *NOCARDIOPSIS PRASINA* EXPRESSED IN *BACILLUS* *LICHENIFORMIS*

*New specifications prepared at the 76<sup>th</sup> JECFA (2012) and published in FAO JECFA Monographs 13 (2012). An ADI “not specified” was established at the 76<sup>th</sup> JECFA (2012)*

## SYNONYMS

Chymotrypsins A and B;  $\alpha$ -chymar ophth; avazyme; chymar; chymotest; enzeon; quimar; quimotrase;  $\alpha$ -chymar;  $\alpha$ -chymotrypsin A;  $\alpha$ -chymotrypsin

## SOURCES

Produced by submerged fermentation of a genetically modified non-pathogenic and non-toxic strain of *Bacillus licheniformis* which contains a gene coding for serine protease with chymotrypsin specificity from *Nocardiaopsis prasina*. The enzyme is secreted to the broth. The cell mass and other solids are separated from the broth by vacuum drum filtration or centrifugation. Ultrafiltration and/or evaporation are applied for concentration and further purification. Residual production strain microorganisms are removed by germ filtration. The final product is formulated using food-grade stabilizing and preserving agents and is standardized to the desired activity.

## Active principles

Serine protease with chymotrypsin specificity

## Systematic names and numbers

EC 3.4.21.1, CAS number: 9004-07-3

## Reactions catalysed

Preferential cleavage: Tyr, Trp, Phe, Leu

## Secondary enzyme activities

None

## DESCRIPTION

Brown liquid

## FUNCTIONAL USES

Enzyme preparation.  
Used in the hydrolysis of proteins like casein, whey, soy isolate, soy concentrate, wheat gluten and corn gluten in the production of partially or extensively hydrolyzed proteins of vegetable and animal origin.

## GENERAL SPECIFICATIONS

Must conform to the current edition of JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

## CHARACTERISTICS

### IDENTIFICATION

Serine protease activity with chymotrypsin specificity

The sample shows serine protease activity with chymotrypsin specificity.

See descriptions under TESTS

### TESTS

Serine protease activity with chymotrypsin specificity

#### Principle:

Serine protease hydrolyses the substrate Suc-Ala-Ala-Pro-Phe-pNA. The release of p-nitroaniline (pNA) results in an increase of absorbance at 405 nm and is proportional to the enzyme activity. Enzyme activity is measured in PROT units. One PROT unit is the amount of enzyme that releases 1 $\mu$ mol of p-nitroaniline from 1 mM substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 9.0 and temperature 37°.

#### Reagents and Solutions:

##### 0.1M Tris buffer, pH 9.0:

Weigh 12.11 g of Tris (tris(hydroxymethyl)aminomethane) and transfer it to a 1L beaker. Weigh out 8.77 g of sodium chloride and transfer to the beaker. Add 900 ml deionized water. Add 3 drops of Triton X-100 while stirring. Maintain buffer temperature between 23 and 25° prior to next step. Measure and adjust pH after all the Triton X-100 has dissolved. Adjust to pH 9.0 $\pm$ 0.1 using 4M HCl. Transfer to a 1L volumetric flask and make up to volume with deionized water. Solution can be stored at room temperature for up to 24 h. Ensure that the buffer is stirred prior to withdrawing for testing.

##### Suc-Ala-Ala-Pro-Phe-pNA (Substrate) Stock Solution:

Weigh 50 mg of the Suc-Ala-Ala-Pro-Phe-pNA substrate in a small beaker. Add 1 ml of DMSO to the substrate. Mix well. Transfer solution to an appropriate container, cover with aluminum foil and store away from light. Solution can be stored at room temperature for up to 1 day.

##### Suc-Ala-Ala-Pro-Phe-pNA (Substrate) Working Solution:

Transfer 350  $\mu$ l of Suc-Ala-Ala-Pro-Phe-pNA (Substrate) Stock Solution into a 25 ml volumetric flask. Make up to volume with 0.1M Tris Buffer, pH 9.0. Mix well, and wrap the flask immediately with foil to avoid light. Solution can be stored at room temperature in the dark, up to 6 h.

##### 10 mM citrate buffer, pH 3.40:

Fill a 1000 ml volumetric with about 500ml deionized water. Weigh

1.56 g of citric acid monohydrate, 0.76 g tri-sodium citrate-dihydrate and 8.77 g sodium chloride and transfer to the volumetric flask. Fill the flask to about 900 ml with deionized water. Stir. Add 3 drops of Triton X-100, and continue to stir. Adjust pH to  $3.40 \pm 0.03$ . if necessary, after the Triton X-100 has dissolved. Make up to volume with deionized water and stir. Solution is stable at room temperature for up to 3 days.

#### Preparation of Standards and Samples:

Preparation of stock standard: Weigh the PROT standard corresponding to 750.1 PROT ( $\pm 0.7$  PROT) in a 250 ml volumetric flask Dissolve and make up to volume with 10 mM citrate buffer. Stir for 15 min at room temperature. This solution can be stored at room temperature for up to 6 h.

Preparation of samples: All samples, liquid or frozen, must be brought to room temperature, and be thoroughly mixed before weighing.

Weigh a known quantity of sample within  $\pm 1$  mg, transfer to an appropriate volumetric flask and make up the volume with 10 mM citrate buffer. The activity of the final dilution(s) of the sample(s) must be around 200 mPROT/ml. Dilute further with citrate buffer, if this concentration is not observed. Solutions can be stored up to 6 h at room temperature.

#### **Procedure**

Preparation of Standard Curve: Prepare a standard curve using the stock standard and 10 mM citrate buffer as shown in the table below. The solutions can be stored up to 6 h at room temperature.

Standard No.	Dilution Ratio	Example		Concentration (mPROT/ml)
		Stock Standard, $\mu$ l	10 mM citrate buffer, $\mu$ l	
1	50	30	1470	60.0
2	30	50	1450	100.0
3	25	60	1440	120.0
4	20	75	1425	150.0
5	15	100	1400	200.0
6	12	125	1375	250.0
7	10	150	1350	300.0

Place the Suc-Ala-Ala-Pro-Phe-pNA (Substrate) Working Solution in a water bath set to  $37.0 \pm 1.0^\circ$ . Set the spectrophotometer at 405 nm and the temperature of the cuvette holder at  $37.0 \pm 0.5^\circ$ . Pipette 2.4 ml of the working substrate solution into a cuvette. Add 600  $\mu$ l of each standard and sample to the cuvette. Place the cuvette in the spectrophotometer set to  $37.0 \pm 0.5^\circ$ . Set and start stopwatch to 1

min. Read absorbance at 20 sec intervals for 3 min.

### Calculations

Calculate the average absorbance per minute for each standard via linear regression. Plot the standard curve using the average absorbance per minute calculated against activity of the standards (mPROT/ml). Read the absorbance of the sample(s) from the standard curve generated and calculate enzyme activity as shown below:

$$\text{Activity, } PROT / g = \frac{S \times V \times F}{W \times 1000}$$

Where, S is reading in mPROT/ml, from the standard curve, V is Volume of the volumetric flask used for the preparation of the sample for the standard curve in ml, F is Dilution Factor (including the 2<sup>nd</sup> dilution, if needed during sample preparation), W is weight of the sample in grams and 1000 is the Conversion Factor from mPROT to PROT.