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Ribonuclease P from *Penicillium citrinum*

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RIBONUCLEASE P FROM *PENICILLIUM CITRINUM*

New specifications prepared at the 92nd JECFA (2021) and published in FAO JECFA Monographs 27 (2021). An ADI of "not specified" was established at the 92nd JECFA (2021).

SYNONYM RNase P

SOURCES Produced by controlled fermentation of a pure culture of non-pathogenic, non-toxigenic strains of *Penicillium citrinum*, either by solid– or liquid–state fermentation techniques. The enzyme is secreted by the microbial cells and is subsequently separated from the resulting cell biomass using a series of filtration steps. The liquid enzyme concentrate is spray dried with food grade dextrin to produce the commercial enzyme preparation.

Active principles: Endonuclease

Systematic names and numbers: Ribonuclease P; EC 3.1.26.5;
CAS No. 71427-00-4

Reaction catalysed: Endonucleolytic cleavage of RNA, removing 5'-nucleotides

Secondary enzyme activities: No significant levels of secondary activities

DESCRIPTION White to dark brown powder

FUNCTIONAL USES Enzyme preparation
Used in the production of processed yeast products and flavouring substances and preparations with naturally occurring RNA.

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

CHARACTERISTICS

IDENTIFICATION

Ribonuclease P activity: The sample shows ribonuclease P activity.
See description under TESTS.

TESTS

METHOD OF ASSAY

Ribonuclease P activity

Principle
Ribonuclease P activity is determined by measuring the production of phosphate that results from the hydrolysis of adenosine 3'-monophosphate, used as a substrate. After a 15-minute reaction, the

absorbance of the reaction mixture is measured spectrophotometrically at 750 nm, enabling the determination of the ribonuclease P activity based on comparison to a disodium hydrogen phosphate standard curve.

One unit of ribonuclease P activity is defined as the quantity of enzyme required to liberate 1 µmol of phosphate (as phosphoric acid) in 1 min at the specified conditions.

Apparatus
Water bath with circulation
UV-Vis spectrophotometer
pH meter
Vortex mixer

Reagents and solutions
- Amidol solution: Dissolve 0.50 g of 2,4-diaminophenol dihydrochloride (Amidol; CAS No. 137-09-47) and 10.0 g of sodium hydrogen sulfite in about 25 ml of deionised water; dilute to 50 ml with deionised water. Filter through medium-porosity filter paper (Toyo Roshi Kaisya, Ltd. grade No.2).
- 1 N Hydrochloric acid: Dilute 98.5 ml of hydrochloric acid (37%) to 1 litre with deionised water.
- 8.5% Sodium chloride solution: Dilute 8.5 g of sodium chloride to 100 ml with deionised water.
- 6% Perchloric acid solution: Dilute 20 ml of perchloric acid (60%) to 200 ml with deionised water.
- Sodium barbital - sodium acetate solution: Dissolve 5.88 g of sodium barbital (sodium 5,5-diethylbarbiturate;
  CAS No. 144-02-5) and 2.34 g of sodium acetate in 100 ml of deionised water; dilute to 200 ml with deionised water.
- Sodium barbital buffer (pH 5.0): Combine 100 ml of Sodium barbital-sodium acetate solution with 40 ml of 8.5% Sodium chloride solution and 200 ml of deionised water. Adjust to pH 5.0 with 1 N Hydrochloric acid. Dilute to 500 ml with deionised water.
- Ammonium heptamolybdate solution:
  Dissolve 8.30 g of ammonium heptamolybdate tetrahydrate (CAS No. 12054-85-2) in about 70 ml of deionised water; dilute to 100 ml with deionised water.
- 0.01 M Disodium hydrogen phosphate standard solution: Dissolve 0.142 g of disodium hydrogen phosphate (CAS No. 7558-79-4) reference material in deionised water; dilute to 100 ml with deionised water (Standard stock solution).

Substrate solution
Dissolve 20 mg of adenosine 3'-monophosphate (CAS No. 84-21-9) in 10 ml of Sodium barbital buffer (pH 5.0). Filter through a PVDF membrane filter (Whatman GD/X PVDF, 0.45 µm).

Sample preparation
Prepare the sample enzyme solution at a dilution expected to catalyse the release of phosphoric acid within the range of the standard curve under

the conditions prescribed. Dissolve an accurately weighed amount of ribonuclease P in deionised water. Prepare serial dilutions as necessary.

Standard curve preparation

Prepare a standard curve as follows.

Set up five 100-ml volumetric flasks and label them Standards 1-5. Dilute aliquots of the Standard stock solution with deionised water as shown in the table below.

Standard No.	Standard stock solution (ml)	[Phosphate] in the volumetric flask, µmol/ml	[Phosphate] in the test tube, µmol/ml
1	1.0	0.1	0.0098
2	5.0	0.5	0.049
3	10.0	1.0	0.098
4	15.0	1.5	0.147
5	20.0	2.0	0.196

1. Transfer 0.5 ml of each of the Standard solutions to individual test tubes.
2. Add 4 ml of 6% Perchloric acid solution to each tube and mix.
3. Add 0.4 ml of the Amidol solution to each tube and mix.
4. Add 0.2 ml of Ammonium heptamolybdate solution to each tube and mix.
5. Measure the absorbance of each solution at 750 nm.
6. Create a standard blank in a sixth test tube in the same manner, using 0.5 ml of water instead of a Standard solution. Subtract the absorbance of the standard blank from the absorbance of each of the Standard solutions. Plot the absorbance of the solutions against the concentration of phosphate (µmol/ml) in the test tube. Determine the slope and y-intercept of the standard curve.

Procedure

For each Sample preparation to be analysed, test in duplicate as follows.

1. Transfer 0.4 ml of the Substrate solution to each of two test tubes and place tubes in a water bath maintained at 70 °C ± 0.5 °C for 5 min.
2. Add 0.1 ml of the Sample preparation to each tube and mix. Incubate the tubes in the water bath at 70°±0.5° for exactly 15 min.
3. At the end of the 15 min reaction period, add 4 ml of 6% Perchloric acid solution to the tubes and mix.
4. Add 0.4 ml of Amidol solution and mix.
5. Add 0.2 ml of Ammonium heptamolybdate solution and mix.
6. Cool the tubes to room temperature and immediately measure the absorbance at 750 nm (Reaction solution).
7. For each Sample preparation to be analysed, prepare duplicate Enzyme blank solutions as follows. Transfer 0.4 ml of the Substrate solution to each of two test tubes, add 4 ml of 6% Perchloric acid solution to the tubes and mix. Add 0.1 ml of the Sample preparation and mix. Add 0.4 ml of Amidol solution and mix. Add 0.2 ml of

Ammonium heptamolybdate solution and mix. Measure the absorbance at 750 nm.

Calculation

Calculate the activity of each sample in U/g, using the average absorbance values for the Reaction solution and the Enzyme blank solution:

$$\text{Ribonuclease P (U/g)} = \frac{[(A_1 - A_2) - b]}{m} x \left[\frac{5.1}{(C\ x\ 0.1\ x\ 15)}\right]$$

Where

A_1 is the absorbance of the Reaction solution.
A_2 is the absorbance of the Enzyme blank solution.
b is the y-intercept of the standard curve.
m is the slope of the standard curve.
5.1 is the total volume of the Reaction solution (ml).
0.1 is the volume of Sample preparation in the Reaction solution (ml).
15 is the reaction time (min).
C is the concentration of enzyme in the Sample preparation (g/ml).