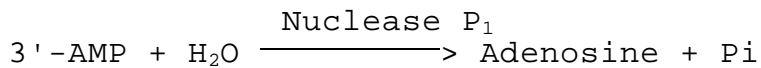


Enzymatic Assay of NUCLEASE P₁
(EC 3.1.30.1)
3'-AMP as Substrate

PRINCIPLE:



Abbreviations used:

3'-AMP = Adenosine 3'-Monophosphate

Pi = Orthophosphate

CONDITIONS: T = 37°C, pH = 7.2, A_{660nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 50 mM Tris HCl Buffer, pH 7.2 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 37°C with 1 M HCl.)
- B. 10 mM Adenosine 3'-Monophosphate Solution (3'-AMP)
(Prepare 5 ml in Reagent A using Adenosine 3'-Monophosphate, Free Acid, Sigma Prod. No. A-9272.)
- C. 10% (w/v) Ammonium Molybdate Solution
(Prepare 25 ml in 10 N H₂SO₄, using Molybdate Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)
- D. Taussky-Shorr Color Reagent (TSCR)
(Prepare by adding 10 ml of Reagent C to 70 ml deionized water. Add 5 g Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water. **STORE IN A DARK CONTAINER; PREPARE FRESH DAILY.**)
- E. Phosphorus Standard (P Std)
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The phosphorus concentration is 20 µg/ml, 0.645 µmoles.)

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REAGENTS: (continued)

- F. 6% (w/v) Perchloric Acid Solution (HClO₄)
 (Prepare 50 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)
- G. Nuclease P₁ Enzyme Solution
 (Immediately before use, prepare a solution containing 0.2 - 0.5 unit/ml in cold deionized water.)

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagent into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.20	0.20
Reagent B (3'-AMP)	0.20	0.20

Equilibrate at 37°C for 5 minutes. Then add:

Reagent G (Enzyme Solution)	0.10	-----
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Incubate at 37°C for exactly 15 minutes. Then add:

Reagent F (HClO ₄)	4.00	4.00
Reagent G (Enzyme Solution)	-----	0.10
Deionized Water	0.50	0.50
Reagent D (TSCR)	5.00	5.00

Mix and incubate for 5 minutes at 25°C. Read the A_{660nm} for both the Test and Blank using a suitable spectrophotometer.

Step 2:

Standard Curve:²

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable container:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	Std
<u>Blank</u>						
Reagent E (P Std)	0.10	0.30	0.50	0.70	1.00	--
Deionized Water	0.90	0.70	0.50	0.30	----	

1.00						
Reagent F (HClO ₄)	4.00	4.00	4.00	4.00	4.00	4.00
4.00						
Reagent D (TSCR)	5.00	5.00	5.00	5.00	5.00	5.00
5.00						

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PROCEDURE: (continued)

Mix by incubate for 5 minutes at 25°C. Read the A_{660nm} for the Standard and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{660nm} \text{ Standard} = A_{660nm} \text{ Standard} - A_{660nm} \text{ Standard Blank}$$

Prepare a standard curve by plotting ΔA_{660nm} Standard vs μmoles of phosphate.

Sample Determination:

$$\Delta A_{660nm} \text{ Test} = A_{660nm} \text{ Test} - A_{660nm} \text{ Blank}$$

Determine the micromoles of Phosphate liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of Phosphate released})(df)}{(15)(0.1)}$$

df = Dilution factor

15 = Time (in minutes) of assay

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of orthophosphate from 3'-AMP per min at pH 7.2 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 0.5 ml reaction mix, the final concentrations are 40 mM Tris, 4 mM adenosine 3'-monophosphate and 0.1 unit nuclease P₁.

REFERENCE:

Fujimoto, M., Kuninaka, A., and Yoshino, H. (1974),
Agricultural Biological Chemistry **38**, 777-783.

NOTES:

1. Glassware must be clean and free of any soap residue, as soap contains phosphates and will contaminate, the assay.
2. TSCR should be added to the samples and standards at the same time and the time interval from TSCR addition to measuring the absorbance on the spectrophotometer must be the same for standards and samples.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.