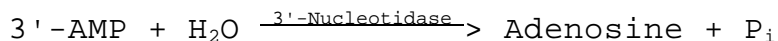


**Enzymatic Assay of 3'-NUCLEOTIDASE
(EC 3.1.3.6)**

PRINCIPLE:



Abbreviations used:

3'-AMP = Adenosine 3'-Monophosphate

P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH = 7.5, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 37°C with 1 M HCl.)
- B. 40 mM Adenosine 3'-Monophosphate Solution (3'-AMP)
(Prepare 5 ml in deionized water using Adenosine 3'-Monophosphate, Sodium Salt, Sigma Prod. No. A-0386.)
- C. 20% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- D. 10% (w/v) Ammonium Molybdate Solution
(Prepare 25 ml in 10 N H₂SO₄ using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)
- E. Taussky-Shorr Color Reagent (TSCR)
(Prepare 100 ml by adding 10 ml of Reagent D to 70 ml of deionized water. Add 5 g Ferrous Sulfate Heptahydrate, Sigma Prod. No. F-0131 and mix until dissolved. Add enough deionized water for a final volume of 100 ml. Store in an amber container.)

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REAGENTS: (continued)

- F. Phosphorus Standard Solution (P Std)
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The phosphorus concentration is 20 µg/ml, 0.645 µmoles/ml.)
- G. 3'-Nucleotidase Enzyme Solution
(Immediately before use, prepare a solution containing 1.5 - 2.5 units/ml of 3'-Nucleotidase in cold Reagent A.)

PROCEDURE:

Step 1:

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reagent A (Buffer) | 1.85 | 1.85 |
| Reagent B (3'-AMP) | 0.10 | 0.10 |

Mix by swirling and equilibrate to 37°C. Then add:

| | | |
|-----------------------------|------|-------|
| Reagent G (Enzyme Solution) | 0.05 | ----- |
|-----------------------------|------|-------|

Immediately mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:

| | | |
|-----------------------------|-------|------|
| Reagent C (TCA) | 0.20 | 0.20 |
| Reagent G (Enzyme Solution) | ----- | 0.05 |

Mix by swirling and centrifuge to clarify.

Step 2:

Pipette (in milliliters) the following reagents into suitable tubes:

| | <u>Test</u> | <u>Blank</u> |
|---------------------------------|-------------|--------------|
| Reagent E (TSCR) | 1.50 | 1.50 |
| Test Supernatant (from Step 1) | 0.30 | ----- |
| Blank Supernatant (from Step 1) | ----- | 0.30 |
| Deionized Water | 1.20 | 1.20 |

**Enzymatic Assay of 3'-NUCLEOTIDASE
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PROCEDURE:

Mix by swirling and incubate at 25°C for 10 minutes.
Transfer the solutions to suitable cuvettes and record the $A_{660\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

A standard curve is made by pipetting (in milliliters) the following reagents into suitable containers:

| | <u>Std 1</u> | <u>Std 2</u> | <u>Std 3</u> | <u>Std 4</u> | <u>Std 5</u> | <u>Std</u> <u>Blank</u> |
|-------------------|--------------|--------------|--------------|--------------|--------------|----------------------------|
| Reagent F (P Std) | 0.10 | 0.25 | 0.35 | 0.60 | 1.00 | --- |
| Deionized Water | 1.40 | 1.25 | 1.15 | 0.90 | 0.50 | 1.50 |
| Reagent E (TSCR) | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |

Mix by inversion and incubate at 25°C for 10 minutes.
Transfer the solutions to suitable cuvettes and record the $A_{660\text{nm}}$ for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

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

Prepare a Standard curve by plotting the $\Delta A_{660\text{nm}}$ of the Standard us μmoles of Phosphate

Sample Determination:

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

Determine the μmoles of Phosphate liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of Phosphate released})(2.2)(\text{df})}{(0.05)(15)}$$

2.2 = Total volume (in milliliters) of Step 1

df = Dilution factor

0.05 = Volume (in milliliter) of enzyme used

15 = Time (in minutes) of assay as per the Unit Definition

**Enzymatic Assay of 3'-NUCLEOTIDASE
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UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of inorganic phosphorus from adenosine 3'-monophosphate per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.0 ml reaction mix the final concentrations are 95 mM Tris, 2 mM adenosine 3'-monophosphate, and 0.075 - 0.125 unit 3'-nucleotidase.

REFERENCE:

Tausky, H.H. and Shorr, E. (1953) *Journal of Biological Chemistry* **202**, 675-685

NOTES:

1. This assay is based on the cited reference.
2. 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.