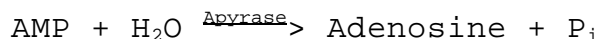


**Enzymatic Assay of APYRASE
(EC 3.6.1.5)
AMP as Substrate**

PRINCIPLE:



Abbreviations:

AMP = Adenosine 5'-Monophosphate

P_i = Inorganic Phosphate

CONDITIONS: T = 30°C, pH 6.5, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 40 mM Succinate Buffer with 4 mM Calcium Chloride, pH 6.5 at 30°C
(Prepare 100 ml in deionized water using Succinic Acid, Free Acid, Prod. No. S-0141, and Calcium Chloride, Dihydrate, Prod. No. C-3881. Adjust to pH 6.5 at 30°C with 1 M KOH.)
- B. 2 mM Adenosine 5'-Monophosphate Solution (AMP)
(Prepare 15 ml in Reagent A using Adenosine 5'-Monophosphate, Sodium Salt, Prod. No. A-1752. Adjust to pH 6.5 at 25°C using 10 mM NaOH.)
- C. 0.1% Albumin Solution (BSA)
(Prepare 50 ml in deionized water using Albumin Bovine Serum, Prod. No. A-4503.)
- D. Apyrase Enzyme Solution
(Immediately before use, prepare a solution containing 5.0 - 10 AMPase units/ml of Apyrase in cold Reagent C.)
- E. Phosphorus Standard Solution
(Use Phosphorus Standard Solution, Prod. No. 661-9. The concentration is, 20 µg/ml, 0.645 µmoles/ml.)

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REAGENTS: (continued)

- F. 10% (w/v) Ammonium Molybdate Solution (Amm. Moly.)
(Prepare 25 ml in 5 M Sulfuric Acid using Molybdic Acid, Ammonium Tetrahydrate Salt, Prod. No. M-0878.)
- G. Taussky-Shorr Reagent (TSCR)
(Prepare by adding 10 ml Reagent F to 70 ml of deionized water. Then add 5 g Ferrous Sulfate, Heptahydrate, Prod. No. F-0131, and mix until dissolved. Add enough deionized water to a final volume of 100 ml.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (AMP)	1.90	1.90

Equilibrate to 30°C. Then add:

Reagent C (BSA)	-----	0.10
Reagent D (Enzyme Solution)	0.10	-----

Immediately mix and incubate at 30°C for exactly 10 minutes. Then add:

Deionized Water	3.00	3.00
Reagent G (TSCR)	5.00	5.00

Mix and incubate at 25°C for 5 minutes.

Transfer to suitable cuvettes and record the $A_{660\text{nm}}$ for both Test and Blank using a suitable spectrophotometer.

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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>
Deionized Water	4.80	4.60	4.40	4.20	4.00	5.00
Reagent E (Phosphorus Std)	0.20	0.40	0.60	0.80	1.00	0.00
Reagent G (TSCR)	5.00	5.00	5.00	5.00	5.00	5.00

Transfer to suitable cuvettes and record the $A_{660\text{nm}}$ for Standards and Blank.

CALCULATIONS:

Find the slope from the plot of the $A_{660\text{nm}}$ of the Standards vs Phosphorus concentration. Use the slope (M) to determine the concentration of the test mixture.

$$\text{Units/mg enzyme} = \frac{A_{660\text{nm}} \text{ Test} - A_{660\text{nm}} \text{ Blank}}{(10) (M) (\text{mg enzyme/RM})}$$

10 = Time of Assay (Unit Definition)

RM = Reaction Mix (volume = 2.00 ml)

UNIT DEFINITION:

One unit will liberate 1.0 μmole of inorganic phosphate from adenosine 5'-monophosphate per minute at pH 6.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 2 ml reaction mix, the final concentrations are 38 mM sodium succinate, 3.8 mM calcium chloride, 1.9 mM AMP, 0.005% BSA, and 0.5 - 1.0 units apyrase.

REFERENCES:

(1953) *J. Biol. Chem.* **202**, 675.

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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