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SIGMA QUALITY CONTROL TEST PROCEDURE

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

**PRINCIPLE:**

$ADP + H_2O \xrightarrow{Apyrase} AMP + P_i$

Abbreviations:
ADP = Adenosine 5'-Diphosphate
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 6.6 at 30°C
(Prepare 100 ml in deionized water using Succinic Acid, Free Acid, Sigma Prod. No. S-7501, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 6.6 at 30°C with 5 M NaOH.)

B. 2.0 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 15 ml in Reagent A using Adenosine 5'-Diphosphate, Di(Monocyclohexylammonium) Salt, Sigma Prod. No. A-4386. Adjust to pH 6.5 at 30°C using 1 M NaOH.)

C. Apyrase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.5 ADPase units/ml of Apyrase in cold deionized water.)

D. Phosphorus Standard Solution
(Use Phosphorus Standard Solution, Sigma Prod. No. 661-9. The concentration is 20 μg/ml, 0.645 μmoles/ml.)

E. 10% (w/v) Ammonium Molybdate Solution (Amm. Moly.)
(Prepare 25 ml in 10 N $H_2SO_4$ using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)

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**REAGENTS:** (continued)

F. Taussky-Shorr Reagent (TSCR)
(Prepare by adding 10 ml of Reagent E to 70 ml of deionized water. Then add a 5 g vial of Ferrous Sulfate, Heptahydrate, Sigma 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:

| | Test | Blank |
|---|---|---|
| Reagent B (ADP) | 1.90 | 1.90 |

Equilibrate to 30°C. Then add:

| | | |
|---|---|---|
| Deionized Water | ------ | 0.10 |
| Reagent C (Enzyme Solution) | 0.10 | ------ |

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

| | | |
|---|---|---|
| Reagent F (TSCR) | 5.00 | 5.00 |
| Deionized Water | 3.00 | 3.00 |

Mix by swirling and immediately (within 1-2 minutes) transfer to suitable cuvettes and record the $A_{660nm}$ for both Test and Blank using a suitable spectrophotometer.

**COLORIMETRIC ASSAY:**

Standard Curve:

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

| | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std Blank |
|---|---|---|---|---|---|---|
| Deionized Water | 4.50 | 4.00 | 3.50 | 3.00 | 2.50 | 5.00 |
| Reagent D (Phosphorus Std) | 0.50 | 1.00 | 1.50 | 2.00 | 2.50 | 0.00 |
| Reagent F (TSCR) | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |

Mix by swirling and immediately (within 1 - 2 minutes) transfer to suitable cuvettes and record the $A_{660nm}$ for Standards and Blank.

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**CALCULATIONS:**

Standard Curve:

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

Prepare a standard curve by plotting $\Delta A_{660nm}$ Standard versus μmoles of Phosphate.

Sample Determination:

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

Determine the micromoles of phosphate liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{\mu\text{moles of Phosphate released})(df)}{(10)(0.1)}$$

df = Dilution factor
10 = Time (in minutes) of assay as per the Unit Definition
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 liberate 1.0 μmole of inorganic phosphate from adenosine 5'-diphosphate per minute at pH 6.5 at 30°C.

**FINAL ASSAY CONCENTRATION:**

In a 2.00 ml reaction mix, the final concentrations are 38 mM succinate, 3.8 mM calcium chloride, 1.9 mM adenosine 5'-diphosphate, and 0.05 - 0.15 unit apyrase.

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**REFERENCES:**


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

Traverso-Cori, A., Chaimovich, H., and Cori, O. (1965) *Archives of Biochemistry and Biophysics* **109**, 173-184


**NOTES:**

1. This assay is based on the cited references.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.