



Product Information

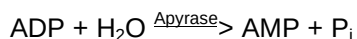
SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of APYRASE

(EC 3.6.1.5)

ADP as Substrate

PRINCIPLE:



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 H₂SO₄ using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)

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

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:

	<u>Test</u>	<u>Blank</u>
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:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
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.

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

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 $\Delta A_{660\text{nm}}$ Standard versus $\mu\text{moles of Phosphate}$.

Sample Determination:

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

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.

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

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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.