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三磷酸腺苷双磷酸酶

PRINCIPLE：

$$ADP + H_2O \xrightarrow{\text{Apyrase}} AMP + Pi$$

Abbreviations:

ADP = Adenosine 5'-Diphosphate

AMP = Adenosine 5'-Monophosphate

Pi = Inorganic Phosphate

CONDITIONS：

T = 30°C, pH 6.5, A660nm, 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, and Calcium Chloride, Dihydrate. 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, Adjust to pH 6.5 at 30°Cusing 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, 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.)

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, 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 A660nm for both Test and Blank using a suitable spectrophotometer.		

COLOR DEVELOPMENT:

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 A660nm for Standards and Blank.

CALCULATIONS:

Standard Curve:

ΔA660nm Standard = A660nm Standard - A660nm Standard Blank

Prepare a standard curve by plotting ΔA660nm Standard versus µmoles of Phosphate.

Sample Determination:

ΔA660nm Test = A660nm Test - A660nm 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

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

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

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.

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 Yuanye Product or Stock numbers are specified, equivalent reagents may be substituted.