SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of PYROPHOSPHATASE, ACID

PRINCIPLE:

$$ATP + H_2O \xrightarrow{\text{Acid Pyrophosphatase}} ADP + P_i$$

Abbreviations:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH 5.0, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 50 mM Sodium Acetate Buffer with 10 mM 2-Mercaptoethanol, 1 mM Ethylenediaminetetraacetic Acid, pH 5.0 at 37°C
(Prepare 25 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, 2-Mercaptoethanol, Sigma Prod. No. M-6250, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.0 at 37°C with 1 M HCl.)

B. 2 mM Adenosine 5'-Triphosphate Solution, pH 5.0 (Substrate Solution)
(Prepare 10 ml in cold Reagent A using Adenosine 5'-Triphosphate, Disodium Salt, Dihydrate, Sigma Prod. No. A-5394.)

C. 10 N Sulfuric Acid Solution (H_2SO_4)
(Prepare 50 ml in deionized water using Sulfuric Acid, Sigma Prod. No. S-1526.)

D. Taussky-Shorr Reagent (TSCR)
(Prepare by adding 10 ml of 10% (w/v) Ammonium Molybdate, Tetrahydrate, Sigma Prod. No. A-7302, in 10 N H_2SO_4, to 70 ml deionized water. Add 5 g of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water.)

REAGENTS: (continued)

E. 0.2 mM Phosphorus Standard (Std)
(Prepare 5 ml in deionized water using Phosphorus Standard Solution, Sigma Stock No. 661-9. The Phosphorus concentration is 20 μg/ml, 0.645 μmoles/ml.)

F. Acid Pyrophosphatase Enzyme Solution
(The enzyme solution should be used undiluted and should be approximately 2000 - 4000 units/ml.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable microcentrifuge tubes:[1]

	Test	Blank
Reagent B (Substrate Solution)	0.045	0.050

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.005	------

Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

Reagent D (TSCR)	0.900	0.900
Deionized Water	0.050	0.050

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

Standard Preparation:

Prepare standards by pipetting (in milliliters) the following reagents into suitable microcentrifuge tubes:

	Std1	Std2	Std3	Std4	Std5	Std Blank
Deionized Water	0.095	0.090	0.080	0.050	------	0.100
Reagent E (Std)	0.005	0.010	0.020	0.050	0.100	------
Reagent D (TSCR)	0.900	0.900	0.900	0.900	0.900	0.900

Mix and incubate at 25°C for 10 minutes. Transfer to suitable cuvettes and record the A_{660nm} for Standards and Standard Blank.

Enzymatic Assay of PYROPHOSPHATASE, ACID

CALCULATIONS:

Standard Curve:

ΔA_{660nm} Standard = A_{660nm} Standard - A_{660nm} Standard Blank

Prepare a standard curve by plotting the ΔA_{660nm} Standard vs nanomoles of Phosphorus.

Sample Determination:

ΔA_{660nm} Sample = A_{660nm} Test - A_{660nm} Test Blank

Determine the nanomoles of Phosphate liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{\text{(nanomoles of Phosphate released)}}{(0.005)(30)}$$

0.005 = Volume (in milliliters) of enzyme used
30 = Time (in minutes) as per the Unit Definition

UNIT DEFINITION:

One unit will release 1.0 nanomole of inorganic phosphorus from ATP in 30 minutes at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 0.050 milliliter reaction mix, the final concentrations are 45 mM sodium acetate, 9 mM 2-mercaptoethanol, 1.8 mM adenosine 5'-triphosphate, 0.9 mM ethylenediaminetetraacetic acid, and 10 - 20 units acid pyrophosphatase.

REFERENCE:

Shinshi, H., Miwa, M., Kato, K., Noguchi, M., Matsushima, T., and Sugimura, T. (1976) *Biochemistry* **15**, 2185-2190.

NOTES:

1. Since very small volumes are utilized, accurate pipetting of solutions is critical.
2. The time period between the addition of Taussky-Shorr reagent and measuring the absorbance in a spectrophotometer must be consistent for all standards and samples.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.