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SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATASE
(EC 3.6.1.3)
Sigma Prod. No. A7510

PRINCIPLE:

$$ATP + H_2O \xrightarrow{ATPase} ADP + P_i$$

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

CONDITIONS: T = 37°C, pH 7.8, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 24 mM Tris HCl Buffer with 0.68 mM Ethylenediaminetetraacetic Acid and 6.0 mM Magnesium Chloride, pH 7.8 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T1503, Ethylenediaminetetraacetic Acid, Free Acid, Sigma Stock No. ED, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M0250. Adjust to pH 7.8 at 37°C with 1 M HCl.)

B. 15 mM Ouabain Solution (Ouabain)
(Prepare 10 ml in Reagent A using Ouabain Octahydrate, Sigma Prod. No. O3125.)

C. 2 M Sodium Chloride Solution (NaCl)
(Prepare 10 ml in deionized water using Sodium Chloride, Sigma Prod. No. S9625.)

D. 45 mM Potassium Chloride and 2 M Sodium Chloride Solution (KCl/NaCl)
(Prepare 10 ml in Reagent C, using Potassium Chloride, Sigma Prod. No. P4504.)

REAGENTS: (continued)

E. 80 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Tris Salt, Sigma Prod. No. A9062. Adjust to pH 7.8 at 37°C with 1 M Tris.)

F. Taussky-Shorr Reagent
(Prepare by adding 10 ml of 10% Ammonium Molybdate, Tetrahydrate Sigma Prod. No. M0878, in 10 N H_2SO_4, to 70 ml deionized water; then add 5 g of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F7002. Bring the volume to 100 ml with deionized water. Store in an amber container.)

G. 20% (w/v) Trichloroacetic Acid (TCA)
(Prepare 100 ml in deionized water using Trichloroacetic Acid Solution, approximately 100% (w/v) 6.1 N, Sigma Stock No. T0699.)

H. Phosphorus Standard (P Std)
(Use Phosphorus Standard Solution, Sigma Stock No. P3869. The concentration is 20 µg/ml, 0.645 µmole/ml.)

I. Adenosine 5'-Triphosphatase Enzyme Solution
(Immediately before use, prepare a solution in cold deionized water containing 0.3 - 0.6 unit/ml.)

PROCEDURE:

Step 1:

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

	Test 1	Blank 1	Test 2	Blank 2
Reagent A (Buffer)	1.25	1.25	1.15	1.15
Reagent B (Ouabain)	----	----	0.10	0.10
Reagent C (NaCl)	----	----	----	----
Reagent D (KCl/NaCl)	0.10	0.10	0.10	0.10
Reagent I (Enzyme Solution)	0.10	----	0.10	----

Mix and equilibrate for several minutes at 37°C. Then add:

Reagent E (ATP)	0.05	0.05	0.05	0.05

PROCEDURE: (continued)

Mix and incubate at 37°C for exactly 15 minutes. Then add:

	Test 1	Blank 1	Test 2	Blank 2
Reagent G (TCA)	1.50	1.50	1.50	1.50

Immediately mix by inversion. Then add:

Reagent I (Enzyme Solution)	----	0.10	----	0.10

Mix by inversion and then centrifuge for 3 minutes to clarify.

Step 2:

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

Reagent F (Taussky-Shorr)	2.00	2.00	2.00	2.00
Test Supernatant	1.00	----	1.00	----
Blank Supernatant	----	1.00	----	1.00
Deionized Water	1.00	1.00	1.00	1.00

Mix and incubate at 25°C for 5 minutes. Read the A_{660nm} for both Tests and Blanks.

Standard Curve:

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

	Std 1	Std 2	Std 3	Blank
Reagent F (Taussky-Shorr)	2.00	2.00	2.00	2.00
Reagent H (P Std)	0.25	0.50	1.00	----
Reagent G (TCA)	0.50	0.50	0.50	0.50
Deionized Water	1.25	1.00	0.50	1.50

Mix and incubate at 25°C for 10 minutes. Read the A_{660nm} for the Standard and Standard Blank using a suitable spectrophotometer.

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CALCULATIONS:

Standard Curve:

$?A_{660nm}$ Standard = A_{660nm} Standard - A_{660nm} Standard Blank

Prepare a standard curve by plotting the $?A_{660nm}$ of the Standard vs µmoles of Phosphate.

Sample Determination:

$?A_{660nm}$ Test = A_{660nm} Test - A_{660nm} Test Blank

Determine the micromoles of Phosphate liberated using the standard curve.

$$\text{Units/ml Protein} = \frac{(\mu\text{moles of Phosphate released})(3.0)(df)}{(15)(0.1)(1.0)}$$

3.0 = Total volume (in milliliters) of Step 1
df = Dilution factor
15 = Time (in minutes) of assay as per the Unit Definition
1.0 = Aliquot of Test Supernatant used in Step 2
0.1 = Volume (in milliliter) of enzyme used

Test 1 = ATPase, activated (Na, K, Mg)
Test 2 = ATPase, not Ouabain sensitive
Ouabain sensitive = Test 1 - Test 2

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

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of inorganic phosphorus from ATP per minute at pH 7.8 at 37°C in the presence of Na^+, K^+, and Mg^{++}.

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FINAL ASSAY CONCENTRATION:

In a 1.50 ml reaction mix, the final concentrations are 20 mM Tris, 0.57 mM ethylenediaminetetraacetic acid, 5 mM magnesium chloride, 3 mM adenosine 5'-triphosphate, 133 mM sodium chloride and 3 mM potassium chloride, 0.03 - 0.06 unit adenosine 5'-triphosphatase and 1 mM ouabain (when present).

REFERENCES:

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

Bonting, S.L., Simon K.A., and Hawkins, N.M. (1961) *Archives of Biochemistry and Biophysics* **95**, 416-423

NOTES:

1. This assay is based on the cited references.

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