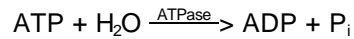


**Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATASE  
(EC 3.6.1.3)**

**PRINCIPLE:**



Abbreviations used:

ATPase = Adenosine 5'-Triphosphatase

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

P<sub>i</sub> = Inorganic Phosphate

**CONDITIONS:** T = 37°C, pH 7.8, A<sub>660nm</sub>, 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. T-1503, Ethylenediaminetetraacetic Acid, Free Acid, Sigma Stock No. ED, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. 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. O-3125.)
- C. 2 M Sodium Chloride Solution (NaCl)  
(Prepare 10 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- 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. P-4504.)

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**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. A-9062. 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. M-0878, in 10 N H<sub>2</sub>SO<sub>4</sub>, to 70 ml deionized water; then add a 5 g vial of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. 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. 490-10.)
  
- H. Phosphorus Standard (P Std)  
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. 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:

	<u>Test 1</u>	<u>Blank 1</u>	<u>Test 2</u>	<u>Blank 2</u>
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
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**PROCEDURE:** (continued)

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

	<u>Test 1</u>	<u>Blank 1</u>	<u>Test 2</u>	<u>Blank 2</u>
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
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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:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Blank</u>
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:

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

Prepare a standard curve by plotting the  $\Delta A_{660\text{nm}}$  of the Standard vs  $\mu\text{moles}$  of Phosphate.

Sample Determination:

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

Determine the micromoles of Phosphate liberated using the standard curve.

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

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  $\mu\text{mole}$  of inorganic phosphorus from ATP per minute at pH 7.8 at 37°C in the presence of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$ .

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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.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**