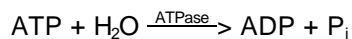


**Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATASE
(EC 3.6.1.3)
From Dog and Rabbit Kidney**

PRINCIPLE:



Abbreviations used:

ATPase = Adenosine 5'-Triphosphatase

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH 7.4, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Tris HCl Buffer, pH 7.4 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.4 at 37°C with 1 M HCl.)
- B. 15 mM Ouabain Solution
(Prepare 10 ml in deionized water using Ouabain, Octahydrate Sigma Prod. No. O-3125.)
- C. 600 mM Potassium Chloride Solution (KCl)
(Prepare 10 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)
- D. 2.5 M Sodium Chloride Solution (NaCl)
(Prepare 10 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- E. 90 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride Hexahydrate, Sigma Prod. No. M-0250.)

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REAGENTS: (continued)

- F. 80 mM Adenosine 5'-Triphosphate (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Tris, Sigma Prod. No. A-9062. Adjust to pH 7.4 at 37°C with 1 M Tris.)
- G. 10% (w/v) Ammonium Molybdate Solution
(Prepare 25 ml in 10 N H₂SO₄ using Molybdic Acid, Ammonia Tetrahydrate Salt, Sigma Prod. No. M-0878.)
- H. Taussky-Shorr Color Reagent (TSCR)
(Prepare by adding 10 ml of Reagent G to 70 ml of deionized water. Add 5 g of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water.)
- I. 20% (w/v) Trichloroacetic Acid (TCA)
(Prepare 100 ml in deionized water using Trichloroacetic Acid, 6.1 N, approximately 100% (w/v), Sigma Stock No. 490-10.)
- J. Phosphorus Standard (P Std)
(Use Phosphorus Standard Solution, Sigma Prod. No. 661-9. The concentration is 20 µg/ml, 0.645 µmoles/ml.)
- K. Adenosine 5'-Triphosphatase Enzyme Solution
(Immediately before use, prepare a solution in cold deionized water containing 4 - 5 units¹/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)	0.90	0.90	0.90	0.90
Reagent B (Ouabain)	-----	-----	0.10	0.10
Reagent C (KCl)	0.05	0.05	0.05	0.05
Reagent D (NaCl)	0.10	0.10	0.10	0.10
Reagent E (MgCl ₂)	0.05	0.05	0.05	0.05
Reagent K (Enzyme)	0.10	-----	0.10	-----
Deionized Water	0.20	0.20	0.10	0.10

Mix and equilibrate for 5 minutes at 37°C. Then add:

Reagent F (ATP)	0.10	0.10	0.10	0.10
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PROCEDURE: (continued)

Mix and incubate at 37°C for exactly 5 minutes. Then add:

	<u>Test 1</u>	<u>Blank 1</u>	<u>Test 2</u>	<u>Blank 2</u>
Reagent I (TCA)	1.50	1.50	1.50	1.50

Immediately mix by inversion. Then add:

Reagent K (Enzyme Solution)	-----	0.10	-----	0.10
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Mix by inversion and then centrifuge in a clinical centrifuge for 3 minutes to clarify.

Step 2:

Pipette (in milliliters) the following into suitable tubes:

Reagent H (TSCR)	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 10 minutes. Read the A_{660nm} for both Tests and Blanks.

Standard Curve:

Prepare a standard curve by pipetting the following into suitable tubes (milliliters).

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Blank</u>
Reagent H (TSCR)	2.00	2.00	2.00	2.00
Reagent J (P Std)	0.25	0.50	1.00	-----
Reagent I (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 each standard using a suitable spectrophotometer.

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CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting $? A_{660\text{nm}} \text{ Standard}$ vs Phosphate concentration.

Sample Determination:

$$? A_{660\text{nm}} \text{ Test}_1 = A_{660\text{nm}} \text{ Test}_1 - A_{660\text{nm}} \text{ Blank}_1$$

$$? A_{660\text{nm}} \text{ Test}_2 = A_{660\text{nm}} \text{ Test}_2 - A_{660\text{nm}} \text{ Blank}_2$$

Determine the micromoles of Phosphate liberated for each Test using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of Phosphate released})(3.0)(\text{df})}{(5)(0.1)(1.0)}$$

1.0 = Aliquot (in milliliter) of Test Supernatant used in Step 2

3 = Total volume (in milliliters) of assay (Step 1)

5 = Time of assay (in minutes) as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used in Step 1

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

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

Test 1 = ATPase, activated (Na, K, Mg)

Test 2 = ATPase, not Ouabain sensitive

Ouabain sensitive = Test 1 - Test 2

UNIT DEFINITION:

One unit will liberate 1.0 μmole of inorganic phosphorus from ATP per minute at pH 7.4 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 30 mM Tris, 3 mM magnesium chloride, 5.3 mM adenosine 5'-triphosphate, 167 mM sodium chloride, 20 mM potassium chloride, 1 mM ouabain (when present), and 0.1 - 0.2 unit ATPase.

REFERENCES:

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

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

NOTES:

1. These units are the fully active (Test 1) units.
2. This assay is based on the cited references.
3. 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.