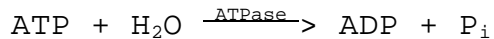


**Enzymatic Assay of ACTOMYOSIN
ATPase Assay**

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate
ATPase = Adenosine 5'-Triphosphatase
ADP = Adenosine 5'-Diphosphate
 P_i = Inorganic phosphate

CONDITIONS: T = 25°C, pH = 9.0, $A_{660\text{nm}}$, Light Path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 200 mM Glycine Buffer, pH 9.0 at 25°C
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 9.0 at 25°C with 1 NaOH.)
- B. 100 mM Calcium Chloride Solution (CaCl_2)
(Prepare 10 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- C. 50 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 5 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- D. 10% (w/v) Ammonium Molybdate Solution
(Prepare 15 ml in 10 N H_2SO_4 using Ammonium Molybdate, Sigma Prod. No. A-7302.)
- E. Taussky-Shorr Reagent (Taussky-Shorr)
(Prepare by adding 10 ml of Reagent D to 70 ml of deionized water, then add 5 g Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water. Store in an amber container.)

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REAGENTS: (continued)

- F. Phosphorus Standard (P Std)
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The concentration is 20 µg/ml, 0.645 µmoles/ml.)
- G. Actomyosin Solution (Actomyosin)
(Immediately before use, prepare a solution containing 2 - 3 mg/ml of Actomyosin in cold deionized water.)

PROCEDURE:

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00
Reagent B (CaCl ₂)	0.20	0.20
Reagent C (ATP)	0.30	0.30
Deionized Water	0.40	0.40

Mix by swirling and equilibrate to 25°C. Then add:

Reagent G (Actomyosin)	0.1	-----
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Mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

Reagent E (Taussky-Shorr)	4.00	4.00
Deionized Water	2.00	2.00
Reagent G (Actomyosin)	-----	0.10

Mix by swirling and incubate at room temperature for approximately 5 minutes. Transfer the solutions to suitable cuvettes and record the A_{660nm} for both the Test and Blank.

Step 2:

Prepare a standard curve by pipetting (in milliliters) the follow reagents into suitable containers:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Blank</u>
Reagent E(Taussky-Shorr)	4.00	4.00	4.00	4.00

Reagent F (P Std)	0.25	0.50	1.00	-----
Deionized Water	3.75	3.50	3.00	4.00

**Enzymatic Assay of ACTOMYOSIN
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PROCEDURE: (continued)

Mix by swirling and incubate at room temperature for approximately 5 minutes. Transfer the solutions to suitable cuvettes and record the $A_{660\text{nm}}$ for both the Standards and Standard Blank.

CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting the $r A_{660\text{nm}}$ Standard vs μmoles of phosphate.

Sample Determination:

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

Determine the micromoles of phosphate liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of phosphate released})(8)(\text{df})}{(0.1)(5)}$$

8 = Total volume (in milliliters) of assay

df = Dilution factor

0.1 = Volume (in milliliter) of Actomyosin used

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

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

UNIT DEFINITION:

One unit will liberate 1.0 micromole of inorganic phosphorus from adenosine 5'-triphosphate per minute at pH 9.0 at 25°C in the presence of calcium.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mixture, the final concentrations are 100 mM glycine, 10 mM calcium chloride, 7.5 mM adenosine 5'-triphosphate, and 0.2 - 0.3 mg actomyosin.

**Enzymatic Assay of ACTOMYOSIN
ATPase Assay**

REFERENCE:

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

Perry, S.V. (1955) *Methods in Enzymology*, II, 582-588

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.