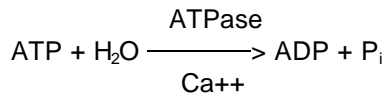


Enzymatic Assay of MYOSIN ATPase Assay

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_{660nm}, 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 (CaCl₂)
(Prepare 10 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod.
No. C-3881.)
- C. 50 mM Adenosine 5'-Triphosphate Solution, pH 6.8 at 25EC (ATP)
(Prepare 5 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod.
No. A-5394. Adjust to pH 6.8 at 25EC with Sodium Carbonate, Anhydrous, Sigma Prod. No.
S-2127.)
- D. 10% (w/v) Ammonium Molybdate Solution
(Prepare 15 ml in 10 N H₂SO₄ using Ammonium Molybdate Tetrahydrate, 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.)

Enzymatic Assay of MYOSIN ATPase Assay

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. Myosin Solution (Myosin)
(Immediately before use, prepare a solution containing 0.50 - 1.00 unit/ml of Myosin in cold deionized water.)

PROCEDURE:

Step 1:

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

	<u>Test 1</u>	<u>Test 2</u>	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00	1.00	1.00
Reagent B (CaCl ₂)	0.20	0.20	0.20	0.20
Reagent C (ATP)	0.30	0.30	0.30	0.30
Deionized Water	0.42	0.41	0.40	0.40

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

Reagent G (Myosin)	0.08	0.09	0.10	-----
--------------------	------	------	------	-------

Mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

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

Mix by swirling and incubate at room temperature for approximately 5 minutes.¹ 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>Std 4</u>	<u>Std 5</u>	<u>Blank</u>
Reagent E(Taussky-Shorr)	4.00	4.00	4.00	4.00	4.00	4.00
Reagent F (P Std)	0.25	0.05	0.75	1.00	1.50	-----
Deionized Water	3.75	3.50	3.25	3.00	2.50	4.00

Enzymatic Assay of Myosin ATPase Assay

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:

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

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

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

df = Dilution factor

0.1 = Volume (in milliliter) of Myosin 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 0.1 unit of ATPase activity.

Enzymatic Assay of Myosin 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. Myosin from Chicken Gizzard, Sigma Prod. No. M-1270, should be read immediately.
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.