

Adenosine 5'-monophosphate, Coenzyme

Catalog	Unit
TBP0093-5G	5 g
TBP0093-25G	25 g

Product Details

Form: Crystalline powder

Molecular Weight: 391.19

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Catalog No.: 208J0000

Unit Definition

1. Triethanolamine buffer, 0.1M, pH 7.6: 1.86 g TEA•HCl in 80 ml distilled water. Adjust to pH 7.6 with 1.0 M NaOH, adjust volume to 100 ml with distilled water.
2. 14mM NADH: 10 mg NADH-Na₂ with 1 ml distilled water.
3. 16.5 mM ATP: 10 mg NADH-Na₂ in 1 ml distilled water.
4. 0.5 M MgSO₄/2 M KCl: 1.23 g MgSO₄• 7 H₂O and 1.49 g KCl in 10 ml distilled water.
5. 32 mM Phosphoenolpyruvate: 15 mg PEP-(CHA)₃ in 1 ml MgSO₄/KCl.
6. LDH, from rabbit muscle: 5 mg protein/ml (550 U/mg).
7. Pyruvate kinase, from rabbit muscle: 10 mg protein/ml (200 U/mg).
8. Myokinase, from rabbit muscle: 5 mg protein/ml (360 U/mg).

Applications

Adenosine 5'-monophosphate (AMP) is used in the determination of creatine kinase and 5'-nucleotidase.

Procedure

1. Dissolve 50 mg AMP in 50 ml distilled water in a volumetric flask.
2. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 340 nm and 25° C.
3. Into two cuvettes, pipette the following:

		BLANK	SAMPLE
Buffer	(1)	3.00 ml	3.00 ml
NADH	(2)	0.05 ml	0.05 ml
ATP	(3)	0.01 ml	0.01 ml
PEP	(5)	0.15 ml	0.15 ml
LDH	(6)	0.01 ml	0.10 ml
PK	(7)	0.02 ml	0.02 ml
Distilled H ₂ O		0.05 ml	- - -
Sample		- - -	0.05 ml

Mix and read the absorbance A₁.
4. Start the reaction by adding 0.01 ml myokinase (8) to both the blank and sample. Read the absorbance A₂.

Calculation

$$\Delta A = (A_1 - A_2)_{\text{sample}} - (A_1 - A_2)_{\text{blank}}$$

$$\text{Total Vol.} = 3.04 \text{ ml}$$

$$449.2 = \text{MW of Adenosine}$$

5'-diphosphate

$$\text{Sample Vol.} = 0.10 \text{ ml}$$

$$\text{Concentration of AMP} = \frac{(3.30) (0.4992)}{(0.63) (1)} \times \Delta A \text{ (mg/ml sample solution)}$$

$$\% \text{ ADP} = \frac{\text{concentration of AMP}}{\text{concentration of sample}} \times 100$$

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