

Adenosine 5'-diphosphate, Coenzyme

Catalog	Unit
TBP0092-1G	1 g
TBP0092-5G	5 g

Product Details

Form: Crystalline powder

Molecular Weight: 507.20

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Assay Method

The assay is based on the reactions described by J.L. Strominger, L.A. Heppel, E.S. Maxwell, Biochim.

Applications

Adenosine 5'-diphosphate (ADP) is used in the determination of creatine kinase, glutamate dehydrogenase, urea and ammonia.

Reagents

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. 0.5 M MgSO₄/2 M KCl: 1.23 g MgSO₄•7H₂O and 1.49 g KCl in 10 ml distilled water.
4. 32 mM PEP: 5 mg PEP-(CHA)₃ in 1 ml solution (3).
5. LDH, from rabbit muscle: 5 mg protein/ml (~550 U/mg).
6. PK, from rabbit muscle: 10 mg protein/ml (200 U/mg).

Procedure

1. Dissolve 50 mg ADP 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 a cuvette, pipette the following:

Buffer	(1)	2.70 ml
NADH	(2)	0.05 ml
PEP	(4)	0.15 ml
LDH	(5)	0.02 ml
sample		0.10 ml
4. Mix and read the absorbance A
5. Start the reaction by adding 0.02 ml PK (6) and read the absorbance A₂.
6. When the reaction is complete add an additional 0.02 ml PK (6). Mix and read the absorbance A₃.

Calculation

$$\Delta A = (A_1 - A_2) - (A_2 - A_3)$$

$$\text{Concentration of nucleotide } (\mu\text{g/ml}) = \frac{(3.04)(0.4492)}{(0.63)(1)} \times \Delta A \text{ (mg/ml sample solution)}$$

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

Total Vol. = 3.04 ml

449.2 = MW of Adenosine 5'-diphosphate

Sample Vol. = 0.10 ml

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