Tribioscience

Adenosine 5'-triphosphate, Coenzyme

Catalog	Unit
TBP0094-1G	1 g
TBP0094-5G	5 g

Product Details

Form: Crystalline powder

Molecular Weight: 605.1

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Unit Definition

1. 0.1M Triethanolamine buffer/substrate, pH 7.6: 1.86 g TEA¿ HCl, 210 mg glycerate-3-P, 125 mg MgSO4¿ 7H2O and 50 mg EDTA with 80 ml distilled water. Adjust pH to 7.6 with 1M NaOH-Na2 and adjust volume to 100 ml with distilled water.
2. 14 mM NADH: 10 mg NADH-Na2 with 1 ml distilled water.
3. Glyceraldehyde-3-phosphate dehydrogenase, from rabbit muscle: 10 mg protein/ml (80 U/mg).
4. 3-Phosphoglycerate kinase, from yeast: 10 mg protein/ml (450 U/mg).

Applications

Adenosine 5'-triphosphate (ATP) is used in the assay of various ATP dependant enzymes (e.g. hexokinase, phosphoglycerate kinase, acetate kinase) and in the enzymatic determination of many metabolites (e.g. glucose, fructose, mannose, maltose, surcose, triglycerides, creatinine, etc.) ATP is also a component of special pharmaceuticals.

Procedure

1. Dissolve 25 mg ATP 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:

		BLANK	SAMPLE
Buffer/subst.	(1)	2.90 ml	2.90 ml
NADH	(2)	0.05 ml	0.05 ml
sample		- - -	0.10 ml
distilled H2O		0.10 ml	- - -

Mix and read absorbance A_1. 4. To both blank and sample add:

GAP-DH	(3)	0.02 ml	0.02 ml
PGK	(4)	0.02 ml	0.02 ml

Mix and read the absorbance A_2 when the reaction is complete.

Calculation

ΔA = (A1 - A2)sample - (A1 - A2)blank

Total Vol. = 3.09 ml
605.2 = MW of Adenosine 5'-diphosphate
Sample Vol. = 0.10 ml

$$\text{Concentration of ATP} = \frac{(3.09)\ (0.507)}{(0.63)\ (1)} \text{ X } \Delta A \text{ (mg/ml sample solution)}$$

$$\text{Concentration of ATP-Na}_2\text{H}_2\bullet 3\text{H}_2\text{O} = \frac{(3.09)\ (0.605)}{(0.63)} \text{ X } \Delta A \text{ (mg/ml sample solution)}$$

$$\%\ \text{ADP} = \frac{\text{concentration of AMP}}{\text{concentration of sample}} \text{ X100}$$

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