Tribioscience

beta-Nicotimamide-Adenine Dinucleotide Phosphate, Reduced, Coenzyme

Catalog	Unit
TBP0097-100MG	100 mg
TBP0097-500MG	500 mg

Product Details

Form: Crystalline powder

Molecular Weight: 833.4

<u>Solubility:</u> Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Purity: 98+%

Catalog No.: 219J0000

Applications

NADPH is used in the determination of leucine aminopeptidase, creatine, ammonia and urea.

Reagents

- 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. 3 M Ammoniumcholride: 16.5 g NH4Cl in distilled water, adjust volume to 100 ml.
- 3. 0.2 M a-Ketoglutarate: 45 mg a-Ketoglutarate-Na2&2H2O in 1 ml distilled water.
- 4. Glutamate dehydrogenase, from bovine liver (20 mg protein/ml): 120 U/mg.

Procedure

- 1. Dissolve 25 mg NADP in 25 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) 2.50 ml
 - NH4Cl (2) 0.15 ml
 - a-KG (3) 0.10 ml

Mix and read the absorbance A1.

- 4. Add 0.10 ml of the sample. Mix and read absorbance A2.
- 5. Start reaction by adding 0.01 ml GLDH. Mix and read absorbance A3.
- 6. Add an additional 0.01 ml GLDH. Mix and read absorbance A4 (absorbance due to enzyme).

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