

Pyruvate Kinase, Enzyme Activity

Catalog	Unit
TBP0085-1KU	1000 U
TBP0085-5KU	5000 U

Product Details

Form: Freeze-dried

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 250 U/mg protein

Protein: 90-95%

Contaminants: Myokinase <0.005%

Glycerol-phosphate dehydrogenase <0.001%

Creatine phosphokinase <0.001%

Adenosine triphosphatase <0.001%

NADH oxidase <0.001%

Phospho-glycerate-mutase <0.001%

Catalog No.: 107A0250

Unit Definition

The amount of enzyme which produces one micromole of pyruvate from phospho(enol)pyruvate, in the presence of ADP, per minute at pH 7.5 and 25°C.

Assay Method

The method of assay has been described by Tietz and Ochoa. The rate of decrease in the absorbancy at 340 nm, resulting from the oxidation of NADH, is proportional to the PK activity.

Applications

Pyruvate kinase (PK) (EC 2.7.1.40) catalyzes the following reaction: Phospho(enol) pyruvate + ADP $\xrightarrow{\text{PK}}$ Pyruvate + ATP

Pyruvate kinase is an important enzyme in glucose metabolism. Mammalian PK of various tissues have distinct characteristics which are related to the specific metabolic requirements of each tissue. PK, prepared from rabbit muscle, is extensively used in the quantitative determination of ADP and of enzymes that catalyze the formation of ADP. It can also be used for the determination of phospho(enol) pyruvate and 2-phosphoglycerate. The assay of PK activity in red blood cells has been described by Tanphaichitr and Van Eys.

Reagents

- 0.06 M Tris-HCl buffer, pH 7.5, in distilled water.
- 0.12 M Magnesium chloride, in distilled water.
- 2.25 M Potassium chloride, in distilled water.
- 0.006 M Adenosine diphosphate (ADP), disodium, in buffer. Prepare fresh.
- 0.043 M Phospho(enol)pyruvate (PEP), trisodium in buffer. Prepare fresh.
- 0.006 M NADH, disodium salt, in buffer. Prepare fresh.
- Lactate dehydrogenase (LDH) solution, 40 U/ml in buffer. Prepare fresh.
- Pyruvate kinase (PK) solution. Using cold 1% bovine serum albumin as solvent, dilute to a final concentration of 0.1-0.2 U/ml. Must be prepared fresh immediately prior to assay.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{340\text{nm}/\text{min}})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})} \quad \text{For research use only}$$