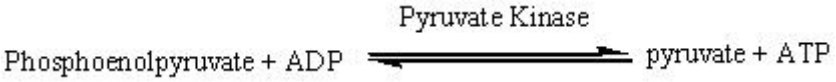
MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 151999, 152000
Pyruvate kinase

Molecular Weight: 237,000 (6)
CAS # : 9001-59-6
E.C. 2.7.1.40
Synonyms: PK; ATP : pyruvate 2-O-phosphotransferase; Phosphoenolpyruvate kinase
Composition: A tetrameric (subunit Molecular Weight 57,000) with four metal binding sites (12). On subunit dissociation, a dimeric intermediate is formed (7). Four sulfhydryl groups have a role at the active site (8,9). There are two binding sites, one for the nucleoside substrate (metal dependent) and one for the acceptor (4).
Reaction:



Source: *Rabbit muscle*
Unit Definition: That amount of enzyme causing the oxidation of one micromole of NADH per minute at 25°C and pH 7.4.
Activators: Has an absolute requirement for a divalent metal ion and a monovalent metal ion such as Mg^{2+} and K^{+}.
Inhibitors: Activity is inhibited by Ca^{2+} (3,13). Fluorophosphate inhibits the enzyme reaction competitively with respect to PEP (14). ATP inhibits the reaction by removal of Mg^{2+} from the substrate $MgADP^{-}$. ATP also appears to inhibit the reaction competitively with respect to both ADP and PEP if the Mg^{2+} concentration is higher than that of ATP (10).
Optimum pH: 7.5
Extinction Coefficient: $E^{1\%}_{280}$ = 5.4 (6).
K_M values (2):

ATP: 0.86 mM
Pyruvate: 10 mM
ADP: 0.3 mM
PEP: 0.07 mM
PK can also utilize other dinucleotide phosphates as substrates including GDP, IDP, dADP, UDP, CDP, dCDP. (11)

Description: A key enzyme in glycogen metabolism.
Assay:
Reagents:

A.0.05 M Imidazole·HCl buffer, pH 7.6, containing 0.12 M potassium chloride and 0.062 M magnesium sulfate.
B.45 mM Adenosine diphosphate
C.45 mM Phosphoenolpyruvate
D.6.6 mM NADH
E.Lactate Dehydrogenase solution: dilute lactate dehydrogenase to a concentration of 1300-1400 units/ml in above imidazole buffer (reagent A). Keep cold during use.
F.Pyruvate Kinase Solution: Dilute immediately before use to obtain a rate of 0.02 - 0.04 DA/minute.

The protein concentration of a solution of the purified enzyme may be determined as follows:

$mg/ml = A_{280} \times 1.85$

Procedure:
Adjust spectrophotometer to 340 nm and 25°C.
Pipette into cuvettes as follows:

Reagent A	2.7 ml

Reagent B	0.1 ml
Reagent D	0.1 ml
Reagent C	0.1 ml
Reagent E	0.01 ml

Mix well and incubate in spectrophotometer for 4-5 minutes to achieve temperature equilibrium and establish blank rate, if any. Add 0.01 ml of diluted enzyme and record decrease in A_{340} for 4-5 minutes. Calculate DA_{340}/minute from the initial linear portion of the curve.
Note: Initial absorbance at 340 nm should be 1.4 ± 0.1. If not, the NADH may be impure or improperly prepared and should not be used.
Calculation:

$$\text{Units/mg} = \frac{\Delta A_{340}/\text{min}}{6.22 \times \text{mg enzyme/ml reaction mixture}}$$

Solubility: Dissolves readily at 5 mg/ml in 0.06M Tris/HCl pH 7.4 to give a clear colorless solution.
Availability:

Catalog Number	Description	Size
151999	Pyruvate Kinase, activity ~350 units/mg protein. Lyophilized powder	1 KU 5 KU 25 KU 50 KU 100 KU
152000	Pyruvate Kinase, activity ~350 units/mg protein. Suspension in 70% saturated ammonium sulfate, pH 6.0	1 KU 5 KU 25 KU 50 KU 100 KU

Also Available:

Catalog Number	Description	Size
152001	Pyruvate Kinase from Bacillus stearothermophilus, lyophilized powder; activity approximately 100-200 units/mg protein	100 U 500 U 1 KU 3 KU
152002	Pyruvate Kinase from porcine heart, lyophilized, activity approximately 200-300 units/mg solid	1 KU 5 KU 10 KU
102031	Imidazole	1 g 5 g 25 g 100 g 250 g
1688045	Hydrochloric acid, 1.0 N solution for titration	10 ml
160053	Adenosine-5'-diphosphate, free acid	100 mg 500 mg 1 g 5 g
195707	Phosphoenolpyruvic acid monopotassium salt	25 mg 100 mg 250 mg 1 g
101168	beta-Nicotinamide Adenine Dinucleotide Reduced (NADH) disodium salt	100 mg 1 g 5 g 10 g
151531	Lactic Dehydrogenase (lactate dehydrogenase) from bovine heart	3 KU 10 KU 25 KU 50 KU 250 KU

References:

– Ainsworth, S., et al., "The regulatory properties of rabbit muscle pyruvate kinase. The influence of substrate concentrations."

Biochem. J., **v. 209(2)**, 401-411 (1983).
– Bergmeyer, H.U. (ed.), *Methods of Enzymatic Analysis, 2nd English Ed.*, **v. 1**, Academic Press: New York, NY, pp. 509-511 (1974).
– Betts, G. and Evans, H., "The effect of cations on the electrophoretic mobility and substrate binding properties of pyruvate kinase." *Biochim. Biophys. Acta*, **v. 167**, 190 (1968).
– Brevet, A., et al., "Muscle pyruvate kinase: interaction with substrates and analogues studied by difference spectroscopy. Comparative studies of the substrate-binding sites of various ATP phosphotransferases." *Biochem.*, **v. 23**, 5241 (1984).
– Bucher, T. and Pfleiderer, G, "Pyruvate kinase from muscle." *Meth. Enzymol.*, **v. 1**, 435-440 (1955).
– Cottam, G., et al., "Subunit structure of rabbit muscle pyruvate kinase." *J. Biol. Chem.*, **v. 244**, 1481 (1969).
– Davies, G. and Kaplan, J., "Use of a diimidoester cross-linking reagent to examine the subunit structure of rabbit muscle pyruvate kinase." *Can. J. Biochem.*, **v. 50**, 416 (1972).
– Flashner, M., et al., "Mechansim of action of pyruvate kinase. Role of sulfhydryl groups in catalytic activity as determined by disulfide interchange." *J. Biol. Chem.*, **v. 247**, 8114 (1972).
– Flashner, M., et al., "Magnetic resonance and catalytic studies of pyruvate kianse with essential sulfhydryl or lysyl epsilon-amino groups chemically modified." *J. Biol. Chem.*, **v. 248**, 3419 (1973).
– Holmsen, H. and Storm, E., "The adenosine triphosphate inhibition of the pyruvate kinase reaction and its dependence on the total magnesium ion concentration." *Biochem. J.*, **v. 112**, 303 (1969).
– Kayne, F.J., in *The Enzymes, 3rd Ed.*, **v. 8**, pt. A, Boyer (ed.), Academic Press: New York, NY, pp. 353-382 (1973).
– Melamud, E. and Mildvan, A., "Magnetic resonance studies of the interaction of Co2+ and phosphoenolpyruvate with pyruvate kinase." *J. Biol. Chem.*, **v. 250**, 8193 (1975).
– Mildvan, A. and Cohn, M., "Kinetic and Magnetic resonance studies of the pyruvate kinase reaction. I. Divalent metal complexes of pyruvate kinase." *J. Biol. Chem.*, **v. 240**, 238 (1965).
– Mildvan, A. and Cohn, M., "Kinetic and magnetic resonance studies of pyruvate kinase. III. The enzyme-metal-phosphoryl bridge complex in the fluorokinase reaction." *Biochem.*, **v. 6**, 1805 (1967).