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SAINT LOUIS, MISSOURI 63178, USA
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TELEPHONE: USA/CANADA 1-800-325-3010
OUTSIDE USA/CANADA call COLLECT 314-771-5750
•
FAX: USA/CANADA 1-800-325-5052
OUTSIDE USA/CANADA 314-771-5757
INTERNET: sigma@sial.com

CREATINE PHOSPHOKINASE FROM RABBIT MUSCLE
Sigma Prod. No. C3755

ACRONYMS: CK and CPK
SYNONYMS: Creatine Kinase

PHYSICAL DESCRIPTION:

Appearance: White to white with a slight yellow cast powder
Molecular Weight: 80,000 to 86,200 depending on reference and method of molecular weight determination.[1,2,3,4]
$E^{1\%}(280)$: 8.76[2]
pH Optimum: pH 8.8-9.0 for the forward reaction and pH 6.0-7.0 for the reverse reaction.[5]
Salts Present: Dialyzed versus 0.01 M Glycine pH 9.0 prior to lyophilization at a protein concentration of approximately 30 mg/ml.

METHOD OF PREPARATION:

Homogenized rabbit muscle is extracted with an aqueous buffer and filtered. The product is precipitated and crystallized in a series of alcohol fractionation steps, dialyzed and lyophilized. No phosphates are used in the preparation. This is a modification of the procedure of Kuby, S. A.[6]

STABILITY / STORAGE AS SUPPLIED:

Stored properly below 0°C, CK should retain its activity for a minimum of two to three years.

SOLUBILITY / SOLUTION STABILITY:

Produces a clear colorless to light yellow solution at 5 mg/ml in 0.25 M glycyl-glycine pH 7.4. Solutions in this buffer at a protein concentration of 1 mg/ml or higher can be stored at -20°C for at least six months. Lower protein concentrations can be supplemented with bovine serum albumin. Unbuffered solutions are less stable and may loose 10% activity in 24 hours at 0-5°C.

STRUCTURE:

Creatine Phosphokinase is a dimer composed predominantly of the skeletal muscle derived homodimer (MM). CK also exists as a heterodimer (MB) particularly in the myocardium. CK derived from brain tissue consists mainly of the brain source homodimer (BB). The amino acid sequences of the M chain and B chains are about 80% homologous. From the sequence, the molecular weight of the M chain is 43,112.[4]

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APPLICATIONS:

CK is a cellular enzyme with a wide tissue distribution. Its physiological role is associated with ATP generation for contractile or transport systems.[7] Increased levels of CK are associated with myocardial infarction, muscular dystrophy, hyperthyroidism, pulmonary infarction and cerebrovascular disease. Variations in relative isozyme distribution can provide additional information in the diagnosis of these conditions. Sigma offers several diagnostic kits for these applications.

SUBSTRATES:

Creatine, N-ethylglycocyamine and glyocyamine have been shown to act as substrates for CK. CK is very specific for ATP/ADP.[8] It has been shown that the Mg^{2+}-ATP complex is the actual substrate for the reaction[9]. Mn^{2+} can substitute for Mg^{2+} [10]. Creatinine, D- and L-arginine, histidine and taurocyamine do not act as substrates.[8] The following Michaelis constants (K^m) were reported in Kuby, S. A., et al.:[5]

ATP = 5×10^{-4} M
creatine = 1.6×10^{-2} M
ADP = 8×10^{-4} M
phosphocreatine = 5×10^{-3} M

INHIBITORS:

ADP is a strong inhibitor of the forward reaction competing with ATP.[8] Divalent cations such as Ca^{2+} (K_i=4.5 mM), Zn^{2+} and Cu^{2+} inhibit CK by competing with Mg^{2+}. Other inhibitors include acetate, acetylsalicylic acid, adenosine, p-aminosalicylic acid, AMP, benzoic acid, bicarbonate, bromide, chloride, p-Chloromercuribenzoic acid, ethylene oxide, 2,4-fluorodinitrobenzene, iodide, malonic acid, NAD, nitrate, phosphate, pyrophosphate, salicylic acid, sulfate, sulfite, thyroxine, trichloroacetate, L-triiodothyroxine, L-triiodothyronine, and tripolyphosphate.[11]

UNIT DEFINITION:

One unit will transfer 1.0 micromole of phosphate from phosphocreatine to ADP per minute at pH 7.4 at 30°C.

REFERENCES:

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4. Pickering, L. et al., *Proc. Nat. Acad. Sci.*, 82, 2310 (1985).
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NOTES: (continued)

7. Watts, D.C., in *The Enzymes*, vol. 8, part A, 3rd ed., Boyer, P.D., ed., (1973).
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10. *Biochemica Information*, Keesey, J, ed., 1st ed, p. 23 (1987).
11. *Handbook of Enzyme Inhibitors*, 2nd ed., Zollner, H, ed, p. 136 (1993).