CUSTOMBIOTECH

Creatine Kinase

Test principle: UV test

Creatine phosphate + ADP $\xrightarrow{\text{creatine kinase}}$ creatine + ATP

ATP + glucose $\xrightarrow{\text{hexokinase}}$ glucose-6-phosphate + ADP

Glucose-6-phosphate + $NADP^+$ $\xrightarrow{\text{G6PDH}}$ 6-phosphogluconate + NADPH + H^+

The assay method using creatine phosphate and ADP was first described by Oliver, modified by Rosalki, and further improved for optimal test conditions by Szasz. Creatine kinase (CK) is rapidly inactivated by oxidation of the sulfhydryl groups in the active center. The enzyme can be reactivated by the addition of acetylcysteine (NAC). Interference by adenylate kinase is prevented by the addition of diadenosine pentaphosphate and AMP. Standardized methods for the determination of CK using the "reverse reaction" and activation by NAC were recommended by the German Society for Clinical Chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC) in 1977 and 1990, respectively. In 2002, the IFCC confirmed their recommendation and extended it to 37°C. The method described here is derived from the formulation recommended by the IFCC.

The rate of NADPH formation is directly proportional to the catalytic CK activity. It is determined by measuring the increase in absorbance at 340 nm.

Proposed reagent composition approximately 3+1 formulation

Reagent 1

Composition	Concentration	Catalog Number
Buffer (Imidazole, pH 6.0)	58 mmol/l	10 034 428 103
N-Acetylcysteine	40 mmol/l	10 068 365 103
EDTA	3 mmol/l	
AMP, di-Na	10 mmol/l	10 000 094 103
Diadenosine pentaphosphate, Tri-Li	24 µmol/l	10 161 624 103
Mg^{2+} (acetate preferred)	20 mmol/l	
D-Glucose	40 mmol/l	
NADP, mono-K	9.5 mmol/l	10 233 536 103
Stabilizer, preservative		

Reagent 2

Composition	Concentration	Catalog Number
EDTA	3 mmol/l	
ADP, mono-K	12 mmol/l	10 233 528 103
N-Methyldiethenolamine	69 mmol/l	
Creatine phosphate, di-Na	180 mmol/l	10 003 506 103
Hexokinase (HK)	>36 kU/l	11 370 600 103
G-6-PDH	>36 kU/l	11 293 206 103
Detergent, preservative, such as Triton X-100 Sodium azide	0.01 %	10 743 119 103

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