COD 11790 1 x 50 mL	COD 11791 4 x 50 mL
STORE AT 2-8ºC	
Reagents for measurement of CK concentration Only for *in vitro* use in the clinical laboratory	

CREATINE KINASE (CK)

BioSystems

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CREATINE KINASE (CK)

IFCC

PRINCIPLE OF THE METHOD

Creatine kinase (CK) catalyzes the phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) coupled reactions[1,2].

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{Glucose - 6 - phosphate}$$

$$\text{Glucose - 6 - phosphate} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{6 - Phosphogluconate} + \text{NADPH} + \text{H}^+$$

CONTENTS

	COD 11790	COD 11791
A. Reagent	1 x 40 mL	4 x 40 mL
B. Reagent	1 x 10 mL	4 x 10 mL

COMPOSITION

A. Reagent: Imidazol 125 mmol/L, EDTA 2 mmol/L, magnesium acetate 12.5 mmol/L, D-glucose 25 mmol/L, N-acetyl cysteine 25 mmol/L, hexokinase 6000 U/L, NADP 2.4 mmol/L, pH 6.7.

DANGER: H360: May damage fertility or the unborn child. P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P280: Wear protective gloves/protective clothing/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P405: Store locked up.

B. Reagent: Creatine phosphate 250 mmol/L, ADP 15 mmol/L, AMP 25 mmol/L, P1,P5-di(adenosine-5'-)pentaphosphate, 102 µmol/L, glucose-6-phosphate dehydrogenase 8000 U/L.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.300 at 340 nm (1 cm cuvette).

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

REAGENT PREPARATION

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B.

Stable for 15 days at 2-8ºC. The working reagent must be protected from light.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 340 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Creatine kinase in serum and plasma is stable for 7 days at 2-8ºC. Use heparin or EDTA as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Sample	50 µL
Working Reagent	1.0 mL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 3 minutes, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The CK concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = \text{U/L}$$

The molar absorbance (ε) of NADPH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05, the sample volume (Vs) is 0.05, and 1 U/L are 16.67 nkat/L.

The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 3333 = U/L x 55561 = nkat/L

REFERENCE VALUES

Reaction Temperature	Men[3] U/L	Men[3] nKat/L	Women[3] U/L	Women[3] nKat/L
37ºC	38-174	633-2900	26-140	433-2334

Children have higher CK concentrations than adults[3]. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 9.2 U/L = 153 nkat/L

– Linearity limit: 1300 U/L = 21671 nkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
175 U/L = 2917 nkat/L	1.8 %	20
567 U/L = 9452 nkat/L	0.7 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
175 U/L = 2917 nkat/L	1.3 %	25
567 U/L = 9452 nkat/L	1.1 %	25

– Sensitivity: 0.3 ΔmA·L/U·min = 5 ΔmA·L/nkat·min

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Bilirubin (< 20 mg/dL) and hemoglobin (< 10 g/L) do not interfere. Lipemia (triglycerides > 5 g/L) interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Creatine kinase (CK) plays an important role in muscle by providing ATP, when muscle contracts, from ADP and using creatine phosphate as the phosphorylation reservoir.

Serum CK originates mainly in muscle and its concentration is subject to a number of physiological variations (sex, age, muscle mass, physical activity and race).

Serum CK concentration is greatly elevated in patients with some diseases of skeletal muscle (muscular distrofy, myositis, polymyositis, malignant hyperthermia, trauma, acute rhabdomyolysis), of the central nervous system (acute cerebrovascular disease, cerebral ischemia, Reye's syndrome) and of the thyroid (hypothyroidism)[3,5].

After a myocardial infarction, CK elevation begins in 3-6 hours and peaks at 24-36 hours. The enzyme is rapidly cleared from the plasma, so that it is common for the activity to return to normality in 3-4 days[3,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37ºC, Part 2. Reference procedure for the measurement of catalytic concentration of creatine kinase. *Clin Chem Lab Med* 2002;40:635-642.
2. IFCC reference procedures for measurement of catalytic concentrations of enzymes: corrigendum, notes and useful advice. *Clin Chem Lab Med* 2010; 48: 615-621.
3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

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