SPECTRUM

The Creative Approach to Bioscience

Creatine Kinase (CK) Optimised DGKC/IFCC Liquid Reagent

REF: 238 000 (5 x 5 ml) 50 Test
REF: 238 001 (6 x 5 ml) 60 Test
REF: 238 002 (6 x 20 ml) 240 Test
REF: 238 004 (6 x 10 ml) 120 Test

Intended Use

Spectrum Diagnostics Creatine Kinase (CK) reagent is intended for the in-vitro quantitative, diagnostic determination of Creatine kinase in human serum on both automated and manual systems.

Background

Creatine kinase (CK) is an enzyme which is found in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarction, acute cerebrovascular disease, trauma or diseases of skeletal muscles. After a myocardial infarction, CK level begins rising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day. CK is present in three different isoenzymatic forms, which could be separated by electrophoresis or column chromatography; each form is originated in different body tissues, paying off their diagnostic determinations. The formula of present reagent is based on DGKC and IFCC recommendations.

Method

Kinetic determination based upon DGKC and IFCC recommendations

Assay Principle

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 (G6PDH) coupled Reactions1,2.

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

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

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

Reagents

Reagent 1 (pH 6.7) (Buffer / Coenzyme)

Imidazol	125 mmol/L
D-Glucose	25 mmol/L
N-Acetyl-L-Cysteine	25 mmol/L
Magnesium acetate	12.5 mmol/L
NADP	2.5 mmol/L
EDTA	2 mmol/L

Reagent 2 (Enzymes)

ADP	15.2 mmol/L
AMP	25 mmol/L
P1,P5-di (adenosine-5'-) penta-phosphate	103 mmol/L
Glucose-6-phosphate Dehydrogenase (G6PDH)	9 KU/L
Creatine phosphate	250 mmol/L
Hexokinase (HK)	3 KU/L

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Storage and Stability

The reagents are stable up to the expiration date specified when stored at 2 – 8 ^{O}C.Once opened, the reagent is stable for 2 months at the specified temperature.

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative	Use by/Expiration Date
IVD For in-vitro diagnostic use	CAUTION. Consult instructions for use
LOT Batch Code/Lot number	Manufactured by
REF Catalogue Number	(Xi) - Irritant
Consult instructions for use	
Temperature Limitation	

Reagent preparation, Storage, and Stability

REF: 238 000 add 1 ml from R2 to one bottle of R1; mix gently
REF: 238 001 add 1 ml from R2 to one bottle of R1; mix gently
REF: 238 002 add 4 ml from R2 to one bottle of R1; mix gently
REF: 238 004 add 2 ml from R2 to one bottle of R1; mix gently

Or prepare the working solution according to the number of test required by mixing 4 volumes of R1 with 1 volume of R2. Stability: 2 weeks at 2-8^{O}C away from light sources.

Specimen Collection and Preservation

Serum free of haemolysis or heparin plasma. Stability 2 days at 20-25 ^{O}C, 7 days at 2-8^{O}C, 4 weeks at -20^{O}C protected from light.

System Parameters

Wavelength	340 nm (334-365 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	Increase
Sample: Reagent Ratio	1:25
e.g.: Reagent volume	1 ml
Sample volume	40 µl
Temperature	37 ^{O}C
Equilibration Time	60 seconds
Read time	1 to 3 minutes
Zero adjustment	against air
Reagent blank limits	
Sensitivity	1 U/L
Linearity	2000 U/L

Procedure

1. Pipette into a thermostatized cuvette:

Working solution	0.5 ml
Serum	20 µL

2. Mix and incubate 60 seconds.
3. Read initial absorbance (A) of the sample, start the stopwatch and read absorbance at 1 minute intervals thereafter for 3 minutes.
4. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

Calculation

$$\Delta A/\text{min} \times 4127 = \text{U/L CK}$$

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

Expected values

Men	24 - 204 U/L
Women	24 - 173 U/L

Quality Control

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characterstics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	86	616
SD	2.41	6.15
CV%	2.8	1.0

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	77	624
SD	1.93	5.0
CV%	2.51	0.8

Methods Comparison

A comparison between Spectrum Diagnostics CK reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.983 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 1 U/L.

Linearity

The reaction is linear up to CK concentration of 2000 U/l; specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

Interferences:

No interferences were observed with haemoglobin until 5 g/L, bilirubin 20 mg/dL and triglycerides 7 mmol/L. Other drugs and substances may interfere[3,4].

References

1. IFCC methods for the measurement of catalytic concentration of enzymes. Part 7: IFCC method for creatine kinase. JIFCC 1989; 1: 130-139.
2. Tietz Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1999.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
238 000	5 x 5 ml
238 001	6 x 5 ml
238 002	6 x 20 ml
238 004	6 x 10 ml

Egyptian Company for Biotechnology (S.A.E)
Obour city industrial area. block 20008 piece 19 A. Cairo. Egypt.
Tel: +202 4489 2248 - Fax: +202 4489 2247
www.spectrum-diagnostics.com
E-mail:info@spectrum-diagnostics.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

CE

TÜV SOUTHWEST CERTIFIED ISO 13485:2016