Cromatest

LINEAR

CREATINE KINASE BR CE

REF 1120010	REF 1120005
1 x 25 mL	2 x 50 mL
CONTENTS	**CONTENTS**
R1. Reagent 1 x 20 mL R2. Reagent 1 x 5 mL	R1. Reagent 4 x 20 mL R2. Reagent 1 x 20 mL
For *in vitro* diagnostic use only	

CREATINE KINASE BR

CK NAC-ACTIVATED

UV enzymatic method

KINETIC

PRINCIPLE

Creatine kinase (CK) catalyzes the reaction between creatine phosphate (CP) and adenosine 5´-diphosphate (ADP) with formation of creatine and adenosine 5´-triphosphate (ATP). The latter phosphorylates glucose to glucose-6-phosphate (G6P) in the presence of hexoquinase (HK). G6P is oxidized to Gluconate-6P in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADP) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6P-DH).
The conversion is monitored kinetically at 340 nm by the rate of increase in absorbance resulting from the reduction of NADP to NADPH proportional to the activity of CK present in the sample.
In this test[1,2] the presence of N-acetilcysteine (NAC) allows the optimal activation of the enzyme.

$$CP + ADP \xrightarrow[pH\ 6.5]{CK\ (AMP,\ NAC)} Creatine + ATP$$

$$ATP + Glucose \xrightarrow{HK} ADP + G6P$$

$$G6P + NADP^+ + H_2O \xrightarrow{G6P\text{-}DH} Gluconate\text{-}6P + NADPH + H^+$$

This test has been formulated according the standarized method described by IFCC. Clin Chem Lab Med 2002; 40(6) : 635-642.

REAGENT COMPOSITION

R1 **Buffer/Glucose/NAC.** Imidazol buffer 100 mmol/L pH 6.7, glucose 20 mmol/L, NAC 20 mmol/L, magnesium acetate 10 mmol/L, NADP 2.5 mmol/L, HK ≥ 4 KU/L, EDTA 2 mmol/L.

R2 **Substrate/Coenzymes.** CP 30 mmol/L, AMP 5 mmol/L, ADP 2 mmol/L, di(adenosine-5´) pentaphosphate 10 µmol/L, G6P-DH ≥ 1.5 KU/L.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm > 0.400 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 1 month at 2-8ºC or 10 days at 16-25ºC.
Protect from light.

SAMPLES

Serum. Stable 8 days at 2-8ºC or 1 month at –20ºC. Chill the samples as rapidily as possible after collection.
Moderately or severely hemolyzed specimens are unsatisfactory for testing, as well as plasmas containing EDTA, heparin, citrate, or fluoride since they may produce unpredictable reaction rates.

INTERFERENCES

- Lipemia (intralipid >5 g/L) may affect the results.
- Bilirubin (< 20 mg/dL), hemoglobin (< 10 g/L), do not interfere.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 25/30/37ºC, capable of reading at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	25ºC	30ºC	37ºC
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample or control	40 µL	40 µL	20 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 3 minutes and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

ΔA/min x 4127 = U/L CK (25/30ºC)
ΔA/min x 8095 = U/L CK (37ºC)

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687

Samples with ΔA/min exceeding 0.270 a 340 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 16.67 = nkat/L

REFERENCE VALUES[1]

Serum

Temperature	25ºC	30ºC	37ºC
Men	≤ 65 U/L (1083 nkat/L)	≤ 105 U/L (1750 nkat/L)	≤ 174 U/L (2900 nkat/L)
Women	≤ 55 U/L (917 nkat/L)	≤ 80 U/L (1334 nkat/L)	≤ 140 U/L (2334 nkat/L)
Children	≤ 94 U/L (1570 nkat/L)	≤ 150 U/L (2500 nkat/L)	≤ 225 U/L (3750 nkat/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of CK. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of CK. Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Creatine kinase (CK) values are high in patients with myocardial infarction, progressive muscular dystrophy, alcoholic myopathy, and delirium tremens, but normal in patients with hepatitis and other forms of liver disease. The high values in patients with hypothyroidism reflect the muscle changes in this condition. Although CK is found almost exclusively in myocardium, muscle, and brain and early reports suggested it to be an almost specific index of injury of myocardium and muscle, more recent reports indicate that, inexplicably high serum CK values can occur in patients with pulmonary infarction and pulmonary edema.

At present, it should be regarded as a useful but not completely specific adjunt in the diagnosis of myocardial and muscle disease. Specificity of CK assay is enhanced by measurement of its isoenzymes.[4-6]

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 10.11 U/L
- ***Linearity :*** Up to 1000 U/L
- ***Precision:***

U/L	Within-run		Between-run	
Mean	227	564	227	564
SD	2.62	13.12	1.15	8.13
CV%	1.15	2.30	2.52	1.44
N	10	10	10	10

- ***Sensitivity :*** 0.125 mA / min/ U/L CK-NAC.
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 25 r = 0.999 y = 1.098x + 6.8

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

1. Szasz, G., Grober, W and Bernt, E. Clin. Chem. 22 : 650 (1976).
2. German Society for Clinical Chemistry: Recommendations of the Enzyme Commision. J. Clin. Chem. Clin. Biochem. 15 : 255 (1977).
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Auvinen, S. Acta. Med. Scand. (Suppl. 539) (1972).
5. Doran, G.R., and Wilkinson, J.H. Clin. Chim. Acta. 62 : 203 (1975).
6. Fisher, M.D., Carliner, N.H., Becker, L.C., Peters, R.W. y Plotnick, G.D. J.A.M.A. 249 : 393 (1983).

B1120-2/0901
R1.ing

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687