Reactivos GPL

Barcelona, España

CE

- CK-MB LQ-

CREATINE KINASE-MB
Inmunoinhibition. Kinetic UV. Liquid

Store at: +2+8ºC.

Presentation:
Cod. EZ008 CONT: R1 1 x 40 mL. + R2 1 x 10 mL.

Procedure

Quantitative determination of creatine kinase-MB (CK-MB).

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
An antibody to the anti CK-M inhibits completely CK-MM and subunit (M) of the CK-MB. The activity of the non-inhibited CK-B subunit is then assayed by the following series of reactions:

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

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

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

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK-B present in the sample[1,2].

COMPOSICIÓN DE LOS REACTIVOS

R 1 Buffer	Imidazol pH 6,10	125 mmol/L
	Glucose	25 mmol/L
	N-acetylcysteine	25 mmol/L
	$NADP^+$	2,52 mmol/L
	Magnesium acetate	12,5 mmol/L
	EDTA	2 mmol/L
	Hexoquinase (HK)	≥6800 U/L
	Anti human CK-M antibody (sheep origin) enough to inhibit up to 2000 U/L of CK-MM	
R 2 Substrate	Imidazol pH 8,90	125 mmol/L
	ADP	15,2 mmol/L
	AMP	25 mmol/L
	di-Adenosine-5- pentaphosphate	103 mmol/L
	Glucosa-6-phosphate deshydrogenase	≥8800 U/L
	Creatinine phosphate	250 mmol/L
CONTROL (Optional)	CK-MB Control: Human liophilyzed serum 1 x 3 mL.	

PRECAUTIONS
R1/R2: H360D- May damage the unborn child. Restricted to professional users.
Follow the precautionary statements given in MSDS and label of the product.

REAGENT PREPARATION AND STABILITY
Working reagent (WR): Mix 1 volume of R2 with 4 volumes of R1.
Stability: 1 day at 20-25ºC or 2 weeks at 2-8ºC.
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm. ≥ 1.20

All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use. Do not use reagents over the expiration date. Do not freeze.

SPECIMEN
Serum free of hemolysis.[1] Samples must be analysed immediately or stability 2 days at 2-8º C or 1 month at -20ºC, protected from light.
CK-MB activity decreses a 10% after 24 hours at 4º C or 1 hour at 25º C.

MATERIAL REQUIRED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25ºC, 30ºC o 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.

General laboratory equipment.

TEST PROCEDURE
1. Assay Conditions
   - Wavelength : ..... 340 nm.
   - Cuvette: ..... 1 cm light path.
   - Constant temperature . . . . .25ºC /30ºC / 37ºC.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a Cuvette(note 1):

WR (mL)	1.0
Sample (µL.)	40

4. Mix and incubate for 10 minutes.
5. Read the absorbance (A_1) of the sample, start the stopwatch and read absorbance at 5 min. (A_2).
6. Calculate the difference of absorbance and the average absorbance difference per minute ($\Delta A = A_2 - A_1$).

CALCULATIONS*(Note 2)*

ΔA x 825* U/L of CK-B

ΔA x 1651* U/L of CK-MB

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 litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to 25ºC	Conversion factor to 30ºC	Conversion factor to 37ºC
25ºC	1.00	1.53	2.38
30ºC	0.65	1.00	1.56
37ºC	0.42	0.64	1.00

QUALITY CONTROL
Controls are recommended to monitor the performance of the procedure, CK-MB CONTROL Ref. QC008. If control values are found outside the defined range, check the instrument and reagents for problems.

Controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES
The suspiction of myocardial damage is based on the three following factors.

	25º C.	30º C.	37º C.
CK-MB	>10 U/L.	> 15 U/L.	> 24 U/L.
TOTAL CK			
Men, up to	80 U/L.	130 U/L.	195 U/L.
Women up to	70 U/L.	110 U/L.	170 U/L.

Percentage of CK-MB activity in sample:

$$\frac{\text{CK – MB Activity}}{\text{CK Total Activity}} \times 100$$: 6-25 % CK-MB Activity in the sample

(These values are for orientation purpose).
It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
CK-MB is an enzyme formed by the association of two subunits from muscle (M) and nerve cells (B). CK-MB is usually present in serum at low concentration; it is increases after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage[5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Measuring Range*:
  From detection limit of 15 U/L. (on Cobas Mira) to linearity limit of 600 U/L., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply result by 10.

Precision:	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	24,95	66	25	74
CV (%)	10,36	4,59	9,80	2,62

- Sensitivity: 6 U/L (on Cobas Mira).
- Accuracy: Results obtained reagents did not show systematic differences when compared with other commercial reagents.
  Correlation coefficient (r): 0.99
  Regression Equation: y=1.0183x + 0.308

The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES
- No interferences were observed with glucose until 7 g/L., haemoglobin until 6 g/L. and triglycerides 8 mmol/L.
- A list of drugs and other interfering substances with CK determination has been reported by Young et. al[3,4].

NOTES
1. Use clean disposable pipette tips for its dispensation.
2. Formulation to reach constant:

ΔA/5 min x 825* or 1651* = U/L CK

$$* \frac{\text{Tv x 1000}}{\varepsilon \text{ x LP x Sv}}$$

Tv= Total volume in mL
ε NADPH = 6.22 at 340 nm
LP= Light path
Sv= Sample volume in mL

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CHEMELEX, S.A.
Pol. Ind. Can Castells. C / Industria 113, Nave J
08420 Canovelles –BARCELONA-
Tel- 34 93 849 17 35 Fax- 34 93 846 78 75