TRUEchemie CK MB TEST KIT

(IFCC)

for the quantitative determination of Creatine Kinase MB in human serum

INTENDED USE

The TRUEchemie CK-MB liquid reagent test kit is used for the quantitative determination of Creatine Kinase MB in human serum.

INTRODUCTION

Creatine Kinase (CK) is an enzyme which consists of isoenzymes mainly of the muscle (CK-M) and the brain (CK-B). CK exists in serum in dimeric form as CK-MM, CK-MB, CK-BB and as macro enzyme. Measurement of CK-MB is a specific test for detection of cardiac muscle damage and therefore is used for diagnosis and monitoring of myocardial infraction. Aspirate and record the absorbance (A1) at 300 seconds after adding the reagent 2 exactly 40 seconds after the first reading record the absorbance (A2) at 30 seconds interval.

PRINCIPLE

CK-MB consists of the subunits CK-M and CK-B. Specific antibodies against CK-M inhibit the complete CK-MM activity (main part of the total CK activity) and the CK-M subunit of CK-MB. Only CK-B activity is measured, which is half of the CK-MB activity. The rate of NADH formation is determined photometrically at 340 nm and is directly proportional to the CK-MB activity in the sample.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CKMB}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK } Mg^{2+}} \text{ADP} + \text{Glucose-6-Phosphate(G-6-P)}$$

$$\text{G-6-P} + \text{NADP Analogue} \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate} + \text{NADP Analogue(Reduced)} + H^{+}$$

PACK SIZE

Kit Size	**2 x 25 ml**
Cat. No.	**ADX351**
Kit Contents	
1) Enzyme Reagent - 1 (R1)	2 x 20 ml
2) Substrate Reagent – 2 (R2)	2 x 5 ml

REAGENTS COMPOSITION

Reagent -1: Clear Colorless liquid.

Glucose	: 20 mmol/L
Magnesium Acetate	: 10 mmol/L
EDTA	: 2.0 mmol/L
ADP	: 2.0 mmol/L
AMP	: 5.0 mmol/L
NADP	: 2.0 mmol/L
N-acetylcysteine	: 20 mmol/L
Anti Human CK-M Antibody	: 2500 U/L

Reagent -2: Clear Colorless liquid.

Creatinine Phosphate	: 30 mmol/L
G6P-DK	: >1.5 U/mL
Di adenosine Penta phosphate	: 10 µmol/L

STORAGE AND STABILITY

The components of the kit, stored at 2-8 °C, will remain stable until the expiry date stated on the label.

REAGENT PREPARATION

Ready to use reagents.

SAMPLE / SPECIMEN AND STORAGE

Collect whole blood by non-traumatic venipuncture and allow it to clot. Centrifuge and remove serum immediately. Serum is reportedly stable for 4 hours at room temperature, 8-12 hours at 4 °C, and 2-3 days when frozen.

Hemolyzed specimens should not be used because of side reactions that may occur due to adenylate kinase, adenosine triphosphate, and glucose-6-phosphate dehydrogenase liberated from red cells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance's at 340 nm

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The disposal of the residues has to be done as per local legal regulations.

TEST PROCEDURE

Wavelength	:	340 nm
Temperature	:	37°C

Prewarm the Reagent to reaction temperature.

	Blank (µl)	Sample (µl)
Distilled Water	1000	--
Enzyme Reagent - 1 (R1)	--	400
Sample	--	25
Mix well and incubate for 5 minutes at 37°C		
Substrate Reagent -2 (R2)	--	100

Mix & take the first reading after 300 Sec. and take THREE additional readings at 30 Sec. intervals. Calculate mean absorbance change per minute (ΔA/min.)

Calculations:
ΔE=Initial absorbance – Absorbance after 300 sec.
Calculations determine the ΔE/min. for every reading and find the mean value.

(Avg ΔE/min.) x 6752 = U/L of CKMB

QUALITY CONTROLS

Control Sera are recommended to monitor the performance of manual and automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

NORMAL VALUES

1. **CK-MB** : upto 24 U/L at 37°C
2. **TOTAL CK**
   Men : < 170 U/L
   Women : < 135 U/L
3. CK-MB activity accounts for 6-25% of the total CK activity
   It is strongly recommended that each laboratory establish its own normal range.

AUTOMATED PROCEDURE

Appropriate Program sheet available for different analyzers upon request.

LIMITATIONS

Sensitivity: 2 IU/L.
Linearity: Up to 750 IU/L.
If the sample concentration above 750 U/L, dilute 1:1 ratio Sample with saline water (NaCl 0.9 %) and re-assayed and the results multiplied by 2.

INTERFERENCES

Certain drugs and medications may affect the activity of CK.
Bilirubin: Less than 10% interference up to 600 µmol/L Bilirubin.
Hemolysis: Less than 10% interference up to 1.25 g/L Hemoglobin.
Lipemia: Less than 10% interference up to 2.5 g/L intralipid.

SYSTEMS PARAMETERS

Mode	:	Kinetic
Factor	:	6752
Wave length	:	340 nm
Units	:	IU/L
Flow cell Temp	:	37°C
Blank	:	Distilled Water
Reagent volume	:	500 µL (R1: 400 µL & R2: 100 µL)
Sample volume	:	25 µL
Lag time	:	300 Sec.
Read time	:	90 Sec.
Normal range	:	upto 24 U/L
Reaction slope	:	Increasing

REFERENCES

1. Stein W. Med Weit. 1985, 36:572 & Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54, 352390 and 974-975
2. GunderWG, NarayananS, WisserH, ZawtaB. List of Anal; Preanal Variables, From the patient to the laboratory. Darmstdt:GIT Verlag 1996.
3. Wurzburg U, Hennrich N, Lang H, Prellwitz W, Neumeier D, Knedel M. Klin Wschr. 1976:54 and 357.
4. Szasz G, Busch EW. Third European Congress of Clinical Chemistry, Brighton England, 3-8 June 1979 (abstract). From the patient to the laboratory. Darmstdt: GIT Verlag 1996.

Index of Symbols

Consult instructions for use	Catalogue number	Use-by date
For *in vitro* diagnostic use only	Batch code	Do not use if package is damaged
Temperature limit 2-8 °C	Keep away from sunlight	Keep dry
Manufacturer	Date of manufacture	
If device is non-sterile	Warnings / Precautions	

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