CK-MB Reagent

Order No.	Description
R85376	10 x 3 mL
R85292	10 x 6.5 mL

INTENDED USE

Cliniqa CK-MB Reagent is intended to measure the activity of the B subunit of the isoenzyme CK-MB of creatine kinase in human serum or plasma employing an in vitro immunoinhibition procedure coupled with a kinetic assay at 340 nm.

CLINICAL SIGNIFICANCE

Creatine kinase (CK) enzymes (EC 2.7.3.2) are dimers formed by the association of two subunits from muscle (M) or nerve cells (B). CK-MM and CK-MB are found mainly in skeletal and heart muscle, while CK-BB is distributed in the brain and smooth muscles.

The major CK activity in normal serum is due to CK-MM. CK-BB is usually present in serum at low concentration. This isoenzyme has been found to be rather unstable. Its concentration increases in brain damage, malignant neoplasms, liver metastases and damage to the pregnant uterus (1). CK-MB is present in low concentration in normal human serum but is increased as a result of heart injury and rarely skeletal muscle damage.

Creatine kinase-MB is generally considered one of the best laboratory indicators of acute myocardial infarction and its detection is of importance in determining the degree of the injury and the efficacy of the treatment.

TEST SUMMARY

Conventional methods for the separation and quantitation of CK isoenzymes have been based on electrophoresis (2) and ion-exchange chromatography (3). While these procedures are useful and suitable for routine use, new methods have recently been introduced by Würzburg et al. (4) and Gerhardt et al. (5, 6). These authors, employing a polyclonal antibody to the CK-M monomer, completely inhibited the activity of CK-MM and one-half the activity of CK-MB. The activity of the non-inhibited CK-B monomer is then assayed.

The sample is incubated in the CK-MB reagent which has been supplemented with anti-CK-M. The activity of the non-inhibited CK-B is then followed by the following series of reactions:

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

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

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-P-DH}} \text{6-PG} + \text{NADPH} + \text{H}^+$$

The increase in absorbance at 340 nm resulting from the formation of reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) is followed with time and is a measure of the enzymatic activity.

The method of assay employed here was thoroughly evaluated by Szasz (7) and its improved formulation (8) has been adopted by the German Society for Clinical Chemistry and by the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Modifications of this formulation, particularly in regard to buffer and adenylate kinase inhibitors, have also been introduced (9). N-acetyl-L-cysteine (NAC) is the thiol compound added to the reagent to rapidly reduce the oxidized sulfhydryl groups in the active site of creatine kinase and thus reactivate the enzyme. Di(adenosine-5') pentaphosphate (AP_5A) (10) and Adenosine-5'-monophosphate (AMP) are used in this formulation to inhibit adenylate kinase.

REAGENT COMPOSITION

The final concentrations of the components in the assay are described below:

Reactive ingredients:

Creatine phosphate	30 mmol/L
Adenosine-5'-diphosphate (ADP)	2 mmol/L
D-glucose	20 mmol/L
Adenosine-5'-monophosphate	5 mmol/L
Nicotinamide Adenine Dinucleotide Phosphate (NADP)	2 mmol/L
Di(adenosine-5')pentaphosphate	10 µmol/L
N-acetyl-L-cysteine	20 mmol/L
Hexokinase (HK)	≥ 3500 U/L
Glucose-6-phosphate dehydrogenase (G-6-P-DH)	≥ 2000 U/L
Anti-human polyclonal CK-M antibody (from sheep or goat) sufficient to inhibit up to 2000 U/L of CK-MM at 37 °C.	

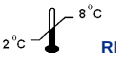
Non-reactive ingredients:

Inhibitors, buffers, stabilizers and fillers

REAGENT PREPARATION

Buffer: Use the Imidazole Buffer solution as provided. This buffer solution is used to reconstitute the CK-MB Reagent prior to use. Use volume specified on the reagent vial label.

Reagent: Dissolve the contents of each vial of CK-MB Reagent (3 mL or 6.5 mL) with the volume of Imidazole Buffer solution as specified on the vial label. Store the reconstituted reagent at 2–8 °C.



REAGENT STORAGE AND STABILITY

The buffer solution is stable until the expiration date on the bottle label when stored at 2–8 °C. Keep bottle tightly capped and guard against contamination.

The dry reagent is stable until the expiration date on the vial label when stored at 2–8 °C.

The reagent reconstituted with the buffer solution is stable for at least six days at 2–8 °C and 24 hours at room temperature (15–25 °C).

If the freshly reconstituted reagent has an absorbance at 340 nm higher than 0.700 against a blank of water, do not use.

If the reagent shows turbidity, do not use.

PRECAUTIONS

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum or plasma can be used for this assay. EDTA is an acceptable anticoagulant.

Avoid exposure of samples to strong light.

Store samples in refrigerator (2–8 °C), but not longer than one week. Freezing of samples (-20 °C) results in minimal loss of activity (11).

INTERFERING SUBSTANCES

Hemolyzed samples should not be used since erythrocytes, contaminants and enzymes will interfere with the assay.

Young (12) lists a number of substances which will affect the determination of creatine kinase.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer capable of accurate absorbance measurements at 340 nm.
2. Matched cuvettes, square,1 cm light path.
3. Constant temperature bath and thermostated spectrophotometer cell compartment.
4. Distilled or deionized water.
5. Physiological saline (sodium chloride: 150 mmol/L in water) to dilute specimen.
6. Pipettes to measure distilled or deionized water, reagents and samples.

MATERIALS PROVIDED

1. Imidazole Buffer
2. CK-MB Reagent

TEST PROCEDURES - MANUAL ASSAYS

1. Determination of total CK activity.
   Determine the total activity of creatine kinase in the samples before carrying out the assay of CK-MB. Use Cliniqa CK Reagents, Cat. Nos. R85191 or R85192. Follow the procedure described in the direction insert for this reagent. For proper comparison with the CK-MB assay, perform the assay of total CK at 37 °C.
   After determining the CK activity, dilute any sample with an activity higher than 2000 U/L at 37 °C, using physiological saline.
2. Determination of CK-B activity.
   Prepare the reagent as directed in the “REAGENT PREPARATION” section.
   A reagent blank is to be performed with the assay.
   Bring needed amount of reagent to approximately 37 °C.

Procedure

1. Pipette 2.0 mL of CK-MB Reagent into a cuvette with 1 cm light path: one cuvette for the test and one cuvette for the blank.
2. Add 100 µL of sample to the test cuvette.
3. Add 100 µL of distilled or deionized water to the blank cuvette.
4. Mix both cuvettes well and incubate at 37 °C for at least five minutes.
5. Measure the absorbance at 340 nm; set spectrophotometer to zero absorbance using water as a reference. Read and record the change in absorbance per minute (ΔA/min.) for three minutes.
6. Subtract the ΔA/min. of the blank (B) from the ΔA/min. of the test (T) to obtain the corrected ΔA/min. (C):
   ΔA/min. (T) - ΔA/min. (B) = ΔA/min. (C)

CALCULATIONS

$$\text{Corrected } \Delta\text{A/min.} \times \frac{\text{TV} \times 1000}{6.22 \times \text{LP} \times \text{SV}} = \text{CK-B Activity (U/L)}$$

Where:

TV = Total volume in mL (2.1 mL)
SV = Sample Volume in mL (0.1 mL)
6.22 = Millimolar extinction coefficient of NADPH
1000 = To convert U/mL to U/L
LP = Light path in cm (1 if a 1 cm cell was used)
Then:

$$\Delta A/min. \times \frac{2.1 \times 1000}{6.22 \times 0.1 \times 1} \text{ x} = \Delta A/min. \text{ x } 3376 = \text{CK-B Activity (U/L)}$$

Multiply CK-B activity (U/L) by 2 to obtain activity in terms of CK-MB (U/L).
Sample Calculation:
ΔA/min. sample = 0.0181
ΔA/min. blank = 0.0001
Corrected ΔA/min. = 0.018
CK-B activity (U/L) = 0.018 x 3376 = 60.7
CK-MB activity = 60.7 x 2 = 121.4 U/L

PERCENTAGE OF CK-MB ACTIVITY IN SAMPLE

This is calculated by dividing the found value of CK-MB activity by the total CK activity and multiplying the ratio by 100 as follows:

$$\frac{\text{CK-MB Activity}}{\text{Total CK Activity}} \times 100 = \% \text{ CK-MB Activity}$$

Sample Calculation:
Total CK activity = 1620 U/L
CK-MB activity = 121 U/L

$$\% \text{ CK-MB Activity} = \frac{121}{1620} \times 100 = 7.5\ \%$$

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Commercially available control materials with established human CK isoenzyme values may be used.

LIMITATIONS OF THE PROCEDURE

1. If the assay of the total CK as reported above is greater than 2000 U/L at 37 °C, dilute the sample appropriately with physiological saline before assay of CK-MB. Multiply the result by the dilution factor to obtain the correct value of the isoenzyme.
2. The method will also measure any CK-BB present in serum, which is usually negligible.

REAGENT PERFORMANCE

1. Correlation: (a) Results obtained in 73 serum samples ranging from 0.16 U/L to 505 U/L were compared with those obtained using a commercial reagent (x) (Sigma). The correlation coefficient was 0.997 and the regression equation was y = 1.02x - 0.65.
   (b) Results obtained in 70 serum samples ranging from 5 U/L to 178 U/L were compared with those obtained using a commercial reagent (x) (Roche Isoscreen®). The correlation coefficient was 0.993 and the regression equation was y = 0.96x- 0.60.
2. Precision:

Within Run			
Mean (U/L)	97.1	53.6	15.8
SD	1.02	0.46	0.31
CV (%)	1.05	0.86	1.96
N	12	12	12
Run to Run			
Mean (U/L)	98.6	57.1	16
SD	1.31	1.42	0.31
CV (%)	1.33	2.49	1.94
N	12	12	12

3. Specificity: The ability of the polyclonal antibody to inhibit the M-subunit of human CK was determined by testing the activity of human CK-MM isoenzyme at a level over 2000 U/L (at 37 °C). The CK-MM isoenzyme was inhibited > 99.5%.
4. Sensitivity: Using this reagent and assay methodology, a change in absorbance per minute (ΔA/min.) of 0.001 read at 340 nm is equivalent to 6.75 U/L of CK-MB activity.

REFERENCE RANGE

The range of values of CK-MB activity in the serum of 120 apparently normal adult males and females has been found to be in our laboratory between 1.1 U/L to 11.6 U/L at 30 °C and 1.8 U/L to 19.4 U/L at 37 °C.

A ratio between CK-MB and Total CK activities above 4% should be considered suspicious, even though it could be caused by extensive skeletal muscle injury. Any ratio above 10% is consistent with acute myocardial infarction (13).

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

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RE-ORDER INFORMATION
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Catalog No.	Catalog No.
REF R85376	REF R85292

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