

CK-MB Isoenzyme Reagent



PRODUCT SUMMARY

Stability	:	7 days at 2-8°C
Measuring Range	:	Up to 1000 U/L
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of buffer.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of CK-MB (CK-2) in human serum.

CLINICAL SIGNIFICANCE^{1,2}

Creatine kinase (ATP: Creatine N-phosphotransferase, EC2.7.3.2) is a dimeric enzyme composed of two types of monomer subunits, M (Muscular) and B (Brain). The subunits combine to form three distinct CK isoenzymes, CK-BB (CK-1), CK-MB (CK-2) and CK-MM (CK-3). CK-MM is the predominant form of CK in skeletal muscle. CK-BB is found in brain and smooth muscle. CK-MB is found in a high concentration in the myocardium (between 14 and 42%) and to a lesser extent skeletal muscle. In the absence of disease, most CK activity in serum is due to the CK-MM isoform.

Damage to the myocardium, as will occur in acute myocardial infarction (AMI), will result in increased circulating levels of the CK-MB isoform. Typically CK-MB levels become elevated 4 to 6 hours after the onset of chest pain, peak between 12 to 24 hours and return to a baseline within 48 hours. Determination of CK-MB usually on admission and at 6 hours, 12 hours, and 24 hours later, is recommended when AMI is suspected.

METHODOLOGY

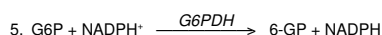
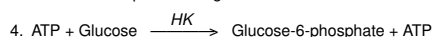
A number of methods are available for the separation and quantification of CK-MB in electrophoresis and immunoinhibition. The immunoinhibition methods have the advantage in that they are easily automated.

The Thermo Scientific CK-MB method utilises an immunoinhibition method. The reagent contains a monoclonal antibody mix to the CK-MB monomer and so completely inhibits the activity of CK-MM and one half the activity of CK-MB. The activity of the non inhibited B monomer subunit of CK-MB is measured which represents half the activity of CK-MB. The method assumes that the activity of CK-BB isoenzyme in serum is essentially zero.

In this method serum is added to a modified CK-NAC reagent which contains the anti M antibody. During the initial incubation the following reactions take place:-

- CK-MM + Antibody \longrightarrow Inhibited CK-MM
CK-MB + Antibody \longrightarrow 50% Inhibited CK-MB
- Inactivated CK-B \longrightarrow NAC \longrightarrow Activated CK-B

The activity of the CK-B is determined using the following reaction sequence:-



Adenylate kinase (Myokinase) is inhibited by AMP and P1P5-diAP



Abbreviations

ADP	=	Adenosine-5'-diphosphate
ATP	=	Adenosine-5'-triphosphate
HK	=	Hexokinase
G-6-P	=	Glucose-6-phosphate
NADP ⁺	=	Nicotinamide Adenine Dinucleotide Phosphate
G-6-PDH	=	Glucose-6-phosphate dehydrogenase
6-PG	=	6-Phosphogluconate
NADPH	=	Reduced NADP
AMP	=	Adenosine-5'-monophosphate
AK	=	Adenylate Kinase
P ¹ P ⁵ -diAP	=	P ¹ P ⁵ -Di(adenosine-5'-)pentaphosphate

REAGENT COMPOSITION

Active Ingredients

Anti human CK-monoclonal antibody mix sufficient to inhibit up to 2000 U/L of CK-M at 37°C

Active Ingredients	Concentration
Imidazole Acetate	100 mmol/L
AMP	5 mmol/L
NADP	2 mmol/L
P ¹ P ⁵ -diAP	10 mmol/L
EDTA	2 mmol/L
Mg Acetate	10 mmol/L
ADP	2 mmol/L
D-Glucose	20 mmol/L
NAC	20 mmol/L
Creatine Phosphate	30 mmol/L
Hexokinase (Yeast)	>3,000 U/L
G-6PDH (Leuconostoc)	>2,000 U/L

Also contains non reactive fillers and stabilisers.

pH 7.00 ± 0.2 at 20°C

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		Reagent A
	Reagent A		Reagent B

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the CK-MB Isoenzyme Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

REAGENT PREPARATION

Reconstitute Reagent A with the volume of buffer, Reagent B, indicated on the vial label. Mix gently until dissolved.

STABILITY AND STORAGE

Prior to use:

When stored refrigerated at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Reconstituted Reagent:

When stored capped at 2-8°C the reagent is stable for at least 7 days.

Indications of Reagent Deterioration:

- Turbidity,
- Absorbance >0.7 at 340 nm (1cm), and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING^{3,4}

Collection: It is recommended that in the case of suspected AMI blood be collected for CK-MB determination on admission and then after 6 hours, 12 hours and 24 hours. The absolute minimum number of samples is 2 obtained 12 and 24 hours after the onset of symptoms.

Serum: Use non haemolysed serum.

Plasma: Not recommended. Heparin, EDTA, fluoride and citrate inhibit CK activity.

Storage: CK is stable for 1 day at 4°C. Stability may vary somewhat for individual serum however, depending on the isoenzyme distribution and patient acid base status. For longer storage store frozen at -20°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30°/37°C
Wavelength	340 nm (334, 365 nm)
Assay Type	Rate/Kinetic
Direction	Increase
Sample :Reagent Ratio	1:20
eg: Sample Vol	10 µL
Reagent Vol	200 µL
Delay/Lag Time	300 seconds
Read Time	300 seconds
Reagent Blank	Low 0.0 AU
(340nm, 1cm lightpath)	High 0.7 AU
Linearity	Up to 1000U/L
Sensitivity	0.15 ΔmAU/min per U/L
(340nm, 1cm lightpath)	

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000}{6.3 \times \text{SV} \times \text{P}} \times 2$$

where:	TV	=	Total reaction volume in mL
	SV	=	Sample volume in mL
	6.3	=	millimolar absorption coefficient of NADH at 340nm (See note 4).
	P	=	Cuvette pathlength in cm.
	2	=	Multiplication of the CK-B value by 2 gives an estimation of the CK-MB activity.

Percentage of CK-MB:

$$\% \text{ CK-MB activity} = \frac{\text{CK-MB U/L}}{\text{Total CK U/L}} \times 100$$

Example: Total CK = 350 U/L
CK-MB = 53 U/L

$$\% \text{ CK-MB activity} = \frac{53 \text{ U/L}}{350 \text{ U/L}} \times 100$$

$$\% \text{ CK-MB activity} = 15\%$$

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- The total CK should be determined first using either the Thermo Scientific IFCC single vial CK reagent or 2 vial IFCC CK reagent. If the change in absorbance is greater than 0.55/min. Repeat the assay with diluted serum. However, the volume fraction of serum in the CK reaction system is critical. Changes in the volume fraction, as will occur in sample predilution, does not produce stoichiometric changes in the reaction rate. If dilution is necessary 150 mmol/L of NaCl is recommended. At a dilution of 1:2 an apparent increase in CK of maximally 10% may be expected.^{5,6} Alternatively, a CK free serum pool can be used for dilution. CK free serum can be produced by heating serum at 56°C for two hours.
- Valid results depend on accurately calibrated instruments, timing and temperature control.
- The millimolar absorption coefficient for NADH at 334 nm = 6.18 and at 365 nm = 3.40.
- Unit conversion U/L x 16.67 x 10⁻³ = µkat/L

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
 - When a new bottle of reagent is used.
 - After preventative maintenance is performed or a critical component is replaced.
- Control results falling above or below the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on an automated clinical chemistry analyser. The following results were obtained:
Haemoglobin: Haemolysed samples should be avoided to minimise interference from adenylate kinase and other reaction intermediates such as ATP and G-6-P.⁷
Bilirubin: No interference from bilirubin up to 340µmol/L (20mg/dL).
Lipaemia: No interference from lipaemia, measured as triglycerides, up to 2.4mmol/L (210 mg/dL).
- CK-BB, if present in the serum is a potential interfering factor in this assay system. Studies have shown that CK-BB only occurs rarely in serum.⁸
- Atypical isoenzymes of CK have also been found to interfere with this assay system. One form, a complex of CK-BB and immunoglobulin G (Macro CK type 1) is more frequently found in elderly women. The presence of atypical CK's does not undermine the value to the assay system as the enzyme pattern over time shows a steady state. In suspected AMI CK-MB values will rise and return to normal levels in 48 hours.⁶
- Young DS has published a comprehensive list of drugs and substances which may interfere with this assay.⁹

EXPECTED VALUES^{10,11}

Total CK	At 37°C	Males	<200 U/L	Females	<180 U/L
	At 30°C	Males	<130 U/L	Females	<113 U/L

CK-MB	At 37°C	<25 U/L
	At 30°C	<16 U/L

CK-MB% A CK-MB ratio between 6 - 25% is consistent with Acute Myocardial Infarction (see Limitation 3).

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

PERFORMANCE DATA

The following performance data was obtained with the CK-MB reagent on a automated Clinical Chemistry system.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure¹².

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	37	156
SD (U/L)	1.7	2.5
CV (%)	4.6	1.6

Between Day:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	37	156
SD (U/L)	1.3	3.3
CV (%)	3.4	2.1

METHOD COMPARISON

Comparison studies were carried out using another commercially available method as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:-

Number of sample pairs	66
Range of sample results	4 - 227 U/L
Mean of reference method results	45 U/L
Mean of CK-MB results	44 U/L
Slope	0.96
Intercept	1.5 U/L
Correlation coefficient	0.999

MEASURING RANGE

When run as recommended, the assay is linear up to 1000 U/L.

SPECIFICITY

Inhibition studies carried out indicate that CK-MB Isoenzyme Reagent effectively inhibited greater than 99% of all CK-MM activity in a sample with 2000 U/L CK-MM.


ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.15 ΔmA/min per U/L.

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REF

Reorder Information

Catalogue No.

REAG A

REAG B

TR14314	20 x 5 mL	1 x 100 mL
TL14301 (iLab 600)	20 x 20 mL	1 x 400 mL