



### ORDER INFORMATION

REF	Kit size
GD5430 00	1x20 + 1x5 ml
KL5430 00	5x16 + 5x4 ml
BK5430 00	3x(16+4 ml)

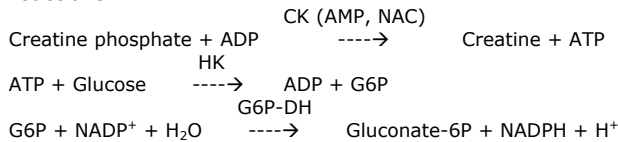
### INDICATION

The enzyme Creatine Kinase (CK) is found mainly in skeletal and heart muscle. It is a dimer formed by the association of two subunits conventionally named M (from *muscle*) and B (from *brain*). The different association of the two subunits is at the base of the differentiation of the three known isoenzymes: MM, MB and BB. An increase of the CK activity may be associated to myocardial infarction, acute cerebrovascular diseases, traumas or diseases involving muscles. After myocardial infarction, CK level begins to increase between the fourth and the sixth hour following the event, reaching a peak between the eighteenth and the thirtieth hour, returning to the normal range during the third day.

CK-MB determination within the proper time frame after infarction is most critical, the useful interval being from about 10 to 24 hour after the infarction. Its detection is of importance in determining the degree of the injury and the efficacy of the treatment.

### PRINCIPLE OF THE METHOD

The immunoinhibition from a specific antibody of both, MM subunits and the single M subunit of CK-MB, allows the determination of the B subunit. The CK-B activity, corresponding to half of CK-MB, is measured by the increasing rate of absorbance resulting from the following reactions:



### COMPOSITION

#### REAGENT A:

Imidazol buffer, pH 6.7	100 mmol/l
N-acetyl cysteine (NAC)	20 mmol/l
Magnesium acetate	10 mmol/l
Glucose	20 mmol/l
NADP	2.5 mmol/l
HK	≥ 4 KU/l

#### REAGENT B:

Creatine phosphate	30 mmol/l
AMP	5 mmol/l
ADP	2 mmol/l
Di(adenosine-5')pentaphosphate	10 μmol/l
G6P-DH	≥ 1.5 KU/l
Sufficient CK-M human antibody to inhibit ≥ 3000 U/l of CK-MM at 37 °C.	

### PREPARATION OF THE REAGENTS

#### Bireagent procedure:

The reagents are liquids ready to use.

#### Monoreagent procedure:

Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g.: 20 ml of RA + 5 ml of RB).

### Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 30 days at 2-8 °C or 5 days at 20-25 °C, protected from light.

Do not utilize the reagent if the absorbance at 340 nm against water is > 0.800, or if the controls values are not inside the declared ranges.

### ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

### SAMPLE

Serum. Stable 8 days at 2-8 °C or 30 days at -20 °C. Chill the samples as rapidly as possible after collection. Avoid using hemolyzed samples.

### Specimen collection / preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

### INTERNAL QUALITY CONTROL

It is recommended to use commercial control sera with known enzymatic activity. Check that the values obtained are within the reference range provided.

### ANALYTICAL PROCEDURE

Working Temperature	37 °C
Wavelength	340 (334-365) nm
Lightpath	1 cm
Type of reaction	Kinetic (increase)

Allow the reagents to reach working temperature before using.

### Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Sample
Reagent B	250 μl	250 μl
Distilled H <sub>2</sub> O	50 μl	-
Sample	-	50 μl
Mix and incubate 2 minutes at 37 °C.		
Then add:		
Reagent A	1000 μl	1000 μl
Mix and incubate 5 minutes at 37 °C.		
Read initial absorbance. Read absorbance again 1, 2, 3 minutes thereafter against Blank. Calculate ΔA/min.		

### Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Sample
Working reagent	1000 µl	1000 µl
Distilled H <sub>2</sub> O	40 µl	-
Sample	-	40 µl

Mix and incubate 5 minutes at 37 °C.  
Read initial absorbance. Read absorbance again 1, 2, 3 minutes thereafter against Blank. Calculate  $\Delta A/\text{min}$ .

### CALCULATION OF RESULTS

CK-B activity (U/l) =  $\Delta A/\text{min} \times 4127$  (37 °C)

CK-MB activity (U/l) = CK-B activity  $\times 2$

### REFERENCE VALUES<sup>(1)</sup>

CK-MB, adults: 2.0 U/l ÷ 19.5 U/l (at 37 °C)

Newborns, infants, and children have higher serum CK-MB values than adults.

A ratio between CK-MB and total CK activities above 4% should be considered suspicious, and above 10% consistent with acute myocardial infarction.<sup>(5)</sup>

Each laboratory is advised to establish the reference interval in relation to its own geographic area.

### ANALYTICAL PERFORMANCES

#### Precision

Within Run (Replicates: 10 for each level)			
n=10	mean (U/l)	SD (U/l)	%CV
Sample 1	16.3	0.32	1.96
Sample 2	56.3	0.64	1.14
Sample 3	96.7	1.07	1.10
Between Run (Replicates: 10 for each level, for 6 days)			
n=10	mean (U/l)	SD (U/l)	%CV
Sample 1	16.1	0.33	2.04
Sample 2	53.5	1.46	2.73
Sample 3	98.5	1.37	1.40

#### Linearity

The assay is linear up to 330 U/l.

#### Sensitivity

Test sensitivity, in terms of limit of detection, is 4 U/l.

#### Correlation

A correlation study comparing the present method and a commercial one gave the following results:

$$y = 0.958x - 0.55 \text{ U/l} \quad r = 0.997$$

#### Interferences

A number of drugs has been listed that will affect the CK determination.<sup>(3)</sup> The present method also measures any CK-BB present in serum, this activity is usually negligible.

### PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

### Waste Management

Please refer to local legal requirements.

### BIBLIOGRAPHY

- Gerhardt and Waldenstrom, G. Clin. Chem. 25 : 1274 (1976).
- Lang, H, and Würzburg, U. Clin. Chem. 28 : 1439 (1982).
- German Society for Clinical Chemistry: Recommendations of the Enzyme Commission. J. Clin. Chem. Clin. Biochem. 15 : 255 (1977).
- Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4 th Edition. AACC Press (1995).
- Wu, A.H.B. and Bowers, G.N, Jr. Clin. Chem. 28, 2017 (1982).
- Stein, W. Med. Welt. 36 : 572 (1985).
- NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
- EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.