



ORDER INFORMATION

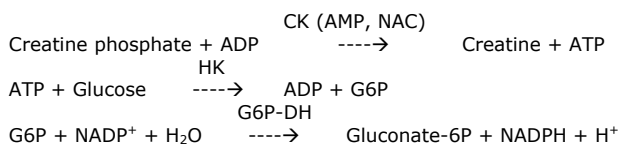
REF	Kit size
GD5432 00	4x16 + 1x16 ml
KL5432 00	5x16 + 5x4 ml
BK5432 00	2x(40+10 ml)

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. CK activity values are high in patients with myocardial infarction, progressive muscular dystrophy, alcoholic myopathy, and delirium tremens, but normal in patients with hepatitis and other forms of liver disease. The high values in patients with hypothyroidism reflect the muscle changes in this condition. Although CK is an almost specific index of injury of myocardium and muscle, more recent reports indicate that inexplicably high serum CK values can occur in patients with pulmonary infarction and pulmonary edema. At present, it should be regarded as a useful but not completely specific adjunct in the diagnosis of myocardial and muscle disease. Specificity of CK assay is enhanced by measurement of its isoenzymes.

PRINCIPLE OF THE METHOD

The CK activity 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
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

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 10 days at 20-25 °C, protected from light.

Do not utilize the reagent if the absorbance at 340 nm against water is > 0.300, 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 ₂ 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 ₂ 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 $\Delta A/\text{min}$.

Note:

Samples with activities higher than 900 U/l should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

CALCULATION OF RESULTS

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

REFERENCE VALUES⁽¹⁾

Test temperature, 37 °C	
CK Male	≤ 174 U/l
CK Female	≤ 140 U/l
Childrens	≤ 225 U/l

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	153	2.4	1.57
Sample 2	546	4.6	0.84
Sample 3	789	10.9	1.40
Between Run (Replicates: 5 for each level, for 5 days)			
n=5	mean (U/l)	SD (U/l)	%CV
Sample 1	153	3.5	2.28
Sample 2	564	3.2	0.56
Sample 3	795	5.7	0.72

Linearity

The assay is linear up to 900 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:

$$y = 1.098x + 6.8 \text{ U/l} \quad r = 0.999$$

Interferences

A number of drugs has been listed that will affect the CK determination.⁽³⁾

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

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 **Manufacturer:**

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