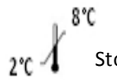
Creatine kinase
Quantitative determination of creatine kinase (CK)

IVD *For in -vitro diagnostic use only*



Store at 2-8°C

INTENDED USE
For the quantitative determination of creatine kinase in human serum or plasma.

INTRODUCTION
Creatine kinase is a cellular enzyme with wide tissue distribution in the body. Its physiological role is associated with adenosine triphosphate (ATP) generation for contractile or transport systems.
Elevated CK values are observed in diseases of skeletal muscle and after myocardial infarction[1,5,6]
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD
Creatine kinase (CK) catalyses the reversible transfer of a phosphate group from phosphocreatine to ADP. This reaction is coupled to those catalysed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH):

Phosphocreatine + ADP —CK→ **Creatine + ATP**

ATP + Glucose —HK→ **ADP + Glucose-6-phosphate**

G6P + NADP —G6P-DH $^{+}$→ **6- Phosphogluconate + NADPH + H^{+}**

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK present in the sample.

REAGENTS

R 1	Imidazol pH 6.7	125mmol/L
	D-Glucose	25mmol/L
	N-Acetyl-L-Cysteine	25mmol/L
	Magnesium acetate	12.5mmol/L
	NADP	2.52mmol/L
	EDTA	2.02mmol/L
	Hexokinase	≥6 800 U/L
R **2**	ADP	15.2mmol/L
	AMP	25mmol/L
	di-Adenosine-5- pentaphosphate	103mmol/L
	Glucose-6-phosphate dehydrogenase (G6P-DH)	≥ 8 800U/L
	Creatine phosphate	250mmol/L

EQUIPMENTS NEEDED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25°C/30°C/ 37°C (± 0.1°C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PREPARATION
Working reagent (WR)
Mix: 4 vol. (R1) + 1 vol. (R2)
Stability: 2 weeks 2-8°C or 48 hours at room temperature.

STORAGE AND STABILITY
- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
- Do not use reagents over the expiration date.
- **Signs of reagent deterioration:**
  - Presence of particles and turbidity.
  - Blank absorbance (A) at 340 nm ≥1.60.

SAMPLES
Serum or plasma[1]: Stability 7 days at 2-8°C, protected from light.
The creatine kinase activity decreases 10% after 1 day at 2-5°C or after 1 hour at 15-25°C.

PROCEDURE
1. Assay conditions:
   Wavelength: ..... 340 nm
   Cuvette :..... 1 cm light path
   Constant temperature ..... 25°C / 30°C / 37°C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	25 - 30°C	37°C
WR (mL)	1.0	1.0
Sample (µL)	40	20

4. Mix, incubate for 2 minutes.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS
25°- 30°C ΔA / min x 4127 = U/L CK
37°C ΔA / min x 8095 = U/L CK
Units:
One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to 25°C	30°C	37°C
25°C	1.00	1.56	2.44
30°C	0.64	1.00	1.56
37°C	0.41	0.63	1.00

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES'

	25°C	30°C	37°C
Men, up to	80 U/L	130 U/L	195 U/L
Women, up to	70 U/L	110 U/L	170 U/L

These values are for orientation purpose; each laboratory should establish its own reference range

PERFORMANCE CHARACTERISTICS
Measuring range:
From detection limit of 1 U/L to linearity limit of 1000 U/L. If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.
Precision:

	Intra-assay		Inter-Assay	
Mean U/L	77	624	83	616
CV(%)	2.50	1.00	2.80	0.80

Sensitivity:
10 U/L on cobas mira.
Accuracy:
Results obtained using reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
No interferences were observed with glucose until 7g/L, hemoglobin until 5g/dL and triglycerides 7mmol/L . A list of drugs and other interfering substances with CK determination has been reported by Young.

REFERENCES

1. Abbot B et al. Creatinine kinase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984: 1112-116.
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3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone number		