# Reactivos GPL

**Barcelona, España**

CE

**- CK-NAC LQ -** CREATINE KINASE
NAC. Kinetic UV. Liquid

*Store at: +2+8ºC.*

*Presentation:*
*Cod.* EZ007 CONT: R1 1 x 40 mL. + R2 x 10 mL.

## Procedure

***Quantitative determination of creatine kinase (CK).***

**Only for *in vitro use in clinical laboratory* (IVD)**

***TEST SUMMARY***

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):

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

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

$$\text{G6P + NADP}^+ \xrightarrow{G6P-DH} \text{6-Phosphogluconate + NADPH + H}^+$$

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK present in the sample[1,2].

***REAGENTS COMPOSITION***

| | | |
|---|---|---|
| **R 1 Buffer** | Imidazol pH 7.0 | 125 mmol/L |
| | Glucose | 25 mmol/L |
| | N-acetyl cysteine | 25 mmol/L |
| | Magnesium acetate | 12.5 mmol/L |
| | $NADP^+$ | 2.5 mmol/L |
| | EDTA | 2 mmol/L |
| | Hexoquinase (HK) | ≥6800U/L |
| **R 2 Substrate** | ADP | 15.2 mmol/L |
| | AMP | 25 mmol/L |
| | di-Adenosine-5- pentaphosphate | 103 mmol/L |
| | Glucose-6-phosphate dehydrogenase (G6P-DH) | ≥8800 U/L |
| | Creatine phosphate | 250 mmol/L |

***REAGENT PREPARATION AND STABILITY***

Working reagent (WR):
Mix 1 volume of R2 with 4 volumes of R1.
Stability: 15 days at 2-8º C or 48 hours at room temperature (15-25º C).
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination 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

**All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8º C. Do not use reagents over the expiration date.**

***SPECIMEN***

Serum free of haemomolysis or heparinized plasma[1]:Stability 7 days at 2-8ºC, protected from light. CK activity decreases 10% after 1 day at 2-8º C. or after 1º hour at 15-25º C.

***MATERIAL REQUIRED BUT NOT PROVIDED***
- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25º C, 30º C o 37º C (± 0.1º C)
- Matched cuvettes 1.0 cm. light path.

**General laboratory equipment.**

***TEST PROCEDURE***

1. **Assay Conditions**
- Wavelength : ..... 340 nm.
- Cuvette: ..... 1 cm light path.
- Constant temperature . . . . . .. . .25ºC /30ºC / 37ºC.

2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a Cuvette(note 1):

| | 25-30ºC | 37ºC. |
|---|---|---|
| WR (mL) | 1.0 | 1.0 |
| Sample (µL.) | 40 | 20 |

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

***CALCULATIONS(Note 2)***

25 – 30º C ΔA/min x 4127* = U/L of CK

37º C. ΔA/min x 8095* = U/L of 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 | | |
|---|---|---|---|
| | 25ºC | 30ºC | 37ºC |
| 25ºC | 1.00 | 1.56 | 2.44 |
| 30ºC | 0.64 | 1.00 | 1.56 |
| 37ºC | 0.41 | 0.63 | 1.00 |

***QUALITY CONTROL***

Control sera are recommended to monitor the performance of the procedure, Control Normal Ref. QC001 and Control Pathological Ref. QC002. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

**Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.**

***REFERENCE VALUES[1]***

| | 30º C | 37º C | 25º 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).
**It is suggested that each laboratory establish its own reference range.**

***CLINICAL SIGNIFICANCE***

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.

***REAGENT PERFORMANCE***
- *Measuring Range*:
  From detection limit of 1 U/L. to linearity limit of 1000 U/L., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply result by 10.
- *Precision*:

| | Intra-assay n= 20 | | Inter-assay n= 20 | |
|---|---|---|---|---|
| Mean (U/L) | 77 | 624 | 83 | 616 |
| CV (%) | 2.50 | 1.00 | 2.80 | 0.80 |

- Sensitivity: 10 U/L = 0.0001 ΔA/min
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
  Correlation coefficient (r): 0.99.
  Regression Equation: y=1.0059x - 1.1072
  The results of the performance characteristics depend on the analyzer used.

***INTERFERING SUBSTANCES***
- No interferences were observed with glucose up to < 7 g/L, triglycerides up to 7 mmol/L and haemoglobin up to 5 g/L[1,2]
- A list of drugs and other interfering substances with CK determination has been reported by Young et. Al[3,4].

***NOTES***
1. Use clean disposable pipette tips for its dispensation.
2. Formulation to reach constant:

ΔA/min x 4127* or 8095* = U/L CK

$$* \frac{Tv \times 1000}{\varepsilon \times LP \times Sv}$$

Tv= Total volume in mL
ε NADPH = 6.22 at 340 nm
LP= Light path
Sv= Sample volume in mL

***BIBLIOGRAPHY***


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.
2. Gerhardt W et al. Creatine kinase B-Subunit activity in serum after immunohinhibition of M-Subunit activity. Clin Chem 1979;(25/7): 1274-1280.
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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