BIOLABO
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CK-NAC IFCC Single Vial

Reagent for quantitative determination of Creatine Kinase activity [EC 2.7.3.2] in human serum.

REF 92207	**R1** 20 x 3 mL	**R2** 1 x 60 mL
REF 92307	**R1** 8 x 20 mL	**R2** 8 x 20 mL

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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

Creatine kinase (CK) is found mainly in cardiac, cerebral and skeletal muscle tissues. So, any dammage or disease of these tissues (myocardial infarction, acute cerebrovascular disease, muscular dystrophy) will result in an increased level of CK activity in serum. There are 3 isoenzymatic forms of CK separable by electrophoresis or column chromatography. Diagnosis specificity may be refined by the determination of the level of each isoforms in serum (variable in conjunction with tissue of origin).
Following myocardial infarction, CK activity begins to rise within 4 to 6 hours, peaks between 18 and 30 hours and returns to normal by the third day.

PRINCIPLE (4) (5) (6)

Enzymatic method described by Oliver and modified by Rosalki and later by Szasz.

1) Creatine phosphate + ADP —**CK**→ Creatine + ATP

2) D-Glucose + ATP —**HK**→ ADP + G-6-Phosphate

3) G-6-Phosphate + NADP+ —**G6-PDH**→ 6-Phosphogluconate + NADPH + H+

The increase in absorbance, proportional to CK activity in the specimen, is measured at 340 nm.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).
- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.

All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

REAGENTS PREPARATION

- **Substrate-Enzymes** (R1):
  Use a non-sharp instrument to remove aluminium cap from the vial.
- **Buffer** (R2): Ready to use
- **Working reagent:**

REF 92307: Transfer promptly the contents of R1 into R2

REF 92207: Transfer promptly 3 mL of R2 into R1.

Mix gently and wait for complete dissolution.

LIMITS (1) (3)

Discard any hemolysed specimen (some enzymes and intermediate products released by erythrocytes interfere with the reaction).
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Thermostated Spectrophotometer or Biochemistry Analyzer

REAGENTS COMPOSITION (7)

R1 SUBSTRATES-ENZYMES SUBS **CK**	**Attention**
Creatine Phosphate	30 mmol/L
D-Glucose	20 mmol/L
N-Acetyl-L-cystein	20 mmol/L
AMP	5 mmol/L
ADP	2 mmol/L
NADP	2 mmol/L
AP5A	10 µmol/L
G-6-PDH (Glucose-6-phosphate dehydrogenase)	> 2500 UI/L
HK (Hexokinase)	> 3000 UI/L

Before reconstitution:
Skin Irrit. 2 : H315 – Causes skin irritation
STOT SE 3 : H335 – May cause respiratory irritation
Acute Tox. 4: H302+H312+H332 – Harmful if swallowed, in contact with skin, if inhaled
Eye Irrit. 2: H319 - Causes serious eye irritation

P280: Wear protective gloves/protective clothing/eye protection/face protection, P264: Wash hands thoroughly after handling, P302+P352 : IF ON SKIN: Wash with soap and water, P304+P340 : IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing, P305+351+338 : IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing, P501: Dispose of contents/container in accordance with dangerous waste regulations. Classification due to: Creatine phosphate 25 - < 50%.

R2 BUFFER BUF **CK**	**Danger**
Imidazole Acetate, pH 6,7	100 mmol/L
EDTA Na2	2 mmol/L
Magnésium Acetate	10 mmol/L
Surfactant, stabilizer	

Repro. 1B : H360 - May damage fertility or the unborn child

P201: Obtain special instructions before use, P202: Do not handle until all safety precautions have been read and understood, P308+P313: IF exposed or concerned: Get medical advice/attention, P405: Store locked up, P501: Dispose of contents/container in accordance with dangerous waste regulations. Classification due to Imidazole < 1%.

For more details, refer to Safety Data Sheet (MSDS)

Once reconstituted: Working Reagent (R1+R2) is classified as contents of vial R2 (Buffer)

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, reagents are stable when stored and used as described in the insert:

Unopened:
- Until expiry date stated on the label of the kit.

Once opened:
- Working reagent (R1+R2) is stable for 1 month
- Discard any reagent if cloudy or if reagent blank is > 0.500 at 340 nm.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Unhemolysed serum. Avoid anticoagulants such as heparin, EDTA, citrate or fluoride. Protect from light and store in an airtight container to prevent loss of CO_2. Adjunction of thiols is not necessary.

CK activity is stable in serum for:
- 4 to 8 h at room temperature, 1 to 2 days at 2-8°C, 1 month at –20°c.

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with | H2O Demineralized water | Biological hazard

CALIBRATION

- REF 95015 BIOLABO Multicalibrator (*traceable to internal Masterlot*)

The calibration frequency depends on proper instrument functions and on the preservation of reagent

EXPECTED VALUES (1) (2)

CK activity in serum is influenced by age, sexe, origin, corpulence, physic activity and other less known genetic factors.

	IU/L (37°C)	µKat/L
Newborn and paediatric	145-1578 (moy 382)	[2,47-26,85]
Alter caesarian births	2-3 x adult	
4 days	3 x adult	
6 weeks-12 years	Adult values	
Adult Men > 19 years	20-200 UI/L	[0,34-3,40]
Adult Women > 19 years	20-180 UI/L	[0,34-3,06]

Heterogeneity of serum CK activities have been described among racial groups ; 97.5th percentiles were respectively as follows:

Population	IU/L
African-American Men	520
Caucasian Men	370
African-American Women	290
Caucasian Women	145

Normal Range can be assay and Instrument dependent.
Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES at 37°C on KENZA 240TX

Linearity Range: between 12 and 800 IU/L

Detection limit: approx. 4 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	63	123.4	556.4
S.D. IU/L	0.7	1.4	3.0
C.V. %	1.1	1.1	0.5

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	65.8	131.2	583.4
S.D. IU/L	2.2	3.8	14.4
C.V. %	3.3	2.9	2.5

Comparison studies with commercially available reagent:
Realised on human specimens (n=100) between 12 and 1063 IU/L

y = 0,9745 x + 4.6059 r = 0.9995

Analytical sensitivity: approx**.** 0.0035 abs/min for 10 IU/L

Interferences:

Total bilirubin	Negative interference from 247 µmol/L
Direct bilirubin	No interference up to 427 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1060 mg/dL
Turbidity	Positive interference from 0.337 abs
Haemoglobin	Positive interference from 257 µmol/L

Other substances may interfere (see § Limits)

On-board stability: Working Reagent is stable 21 days

Calibration Frequency: 14 days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 657-666, 728, 1185-1190.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 306-309*
(3) *p .3-185 to 3-190.*
(4) *Oliver I.T., Biochem J., **61**, (1955), p.116-122.*
(5) *Rosalki S.B., J. Lab. Clin. Med., **69**, (1967), p.696-705.*
(6) *Szasz G., Gruber W., and Bernt E., Clin. Chem., **22** (1976), p.650-656.*
(7) *Horder M and al, Approved IFCC recommendation on methods for the measurement of catalytic concentration of enzymes. Part 7. IFCC method for creatine kinase [EC 2. 7. 3. 2]. Eur J. clin. Chem. Clin. Biochem., 29, p435-456 (1991).*

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO EXATROL-P Level II
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

PROCEDURE

Detailed Kenza 240TX procedure is available on request

Wavelength: 340 nm
Temperature: 37°C
Let stand reagent and specimen at room temperature.

	Automated analyzer	Manual procedure
Reagent	240 µL	1000 µL
Calibrator/Control or Specimen	12 µL	50 µL
Mix. Start a timer and record initial absorbance at 340 nm after 2 minutes. Record the absorbance again every minute during 3 minutes.		
Calculate absorbance change per minute (ΔAbs/min).		

Notes:

1- Performances and stability data's have been validated on KENZA 240TX and KENZA 450TX.

2-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user.

3- Applications proposal are available on request

CALCULATION

Calculate the result as follows:

With seric multicalibrator:

$$\text{CK Activity} = \frac{(\Delta\text{Abs/min) Assay}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Concentration}$$

With theoretical factor :

Activity (IU/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{VR \times 1000}{6.22 \times VE \times P}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
6.22 = Molar extinction coefficient for NADPH at 340 nm
P = Pathlength (cm).

Example, with manual Procedure,
(Path length 1 cm, 37°C, 340 nm):

IU/L = (ΔAbs/min) x 3333

$$\text{µKat/L} = \frac{\text{UI/L}}{60}$$