CK-NAC – DGKC and IFCC method
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182566	n° 10 vials x 8 mL (R.A) n° 2 vials x 10 mL (R.B)
	REF B75182567	n° 8 vials x 2,4 mL (R.A) n° 1 vials x 5 mL (R.B)
CHEMILAB	REF B81180231	n° 2 vials x 32 mL (R.A) n° 2 vials x 8 mL (R.B)

INTENDED USE
Quantitative in vitro determination of the concentration of Total Creatine phospho kinase (CK-NAC) in serum and plasma. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE
Creatine kinase (CK) is an enzyme made up of isoenzymes – mainly muscular (CK-M) – and cerebral tissue (CK-B). CK exists in the human body in dimeric form as CK-MM, CK-MB and CK-BB and as macro-enzymes. Measurement of CK-NAC is a specific test to individuate damage to the heart muscle and is utilized for diagnosis and monitoring of myocardial infarction.

PRINCIPLE OF THE METHOD
UV test UV optimized according to DGKC (German Society of Clinical Chemistry) and IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) specifications.

$$CreatinePhosphate + ADP \xrightarrow{CK} Creatine + ATP$$

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

$$Glucose-6-Phosphate + NADP^{+} \xleftrightarrow{G6P-DH} 6-phosphoglu conolactone + NADPH + H^{+}$$

Storage and stability
= **Storage temperature 2-8 °C**

If stored closed at the indicated temperature, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentration

Reagent A			
	Conc.	U.M.	
Imidazole buffer pH 6.5	60	mmol/L	
Glucose	27	mmol/L	
N - Acetylcysteine	27	mmol/L	
Magnesium Acetate	14	mmol/L	
EDTA-Na_2	2	mmol/L	
NADP	2.7	mmol/L	
Hexokinase	≥ 5	kU/L	
Reagent B			
Imidazole buffer	160	mmol/L	
ADP	11	mmol/L	
AMP	28	mmol/L	
Glucose-6 phosphate dehydrogenase	≥ 14	kU/L	
Di-Adenosine Pentaphosphate	55	µmol/L	
Creatine phosphate	160	mmol/L	
EDTA-Na_2	2	mmol/L	

***1 - GHS05**

***2 - GHS07 – GHS08**

***1** _ *Warning:* **DANGER**
Contains: Imidazole (CAS 288-32-4)
H360D – May damage the unborn child.
P201 - Obtain special instructions before use.
P202 - Do not handle until all safety precautions have been read and understood.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313 - IF exposed or concerned: Get medical advice/attention.
P501 - Dispose of contents/container in accordance with local /regional /national/ international regulations.
*2 _*Warning:* **DANGER**
Contains: Imidazole (CAS 288-32-4)
H360D – May damage the unborn child.
H315 - Causes skin irritation.
H319 - Causes severe eye irritation**.**
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P313 - IF exposed or concerned: Get medical advice/attention.
P321 - Specific treatment (see on this label).
P332+P313 - If skin irritation occurs: Get medical advice/attention.
P501 - Dispose of contents/container in accordance with local/ regional/ national/ international regulations.

Reagents included in the kit
The reagent is described above.
Materials required but not supplied in the kit
Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS
1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements.
Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PROCEDURE
Quality control
Control sera with a known titer of CK-NAC are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration
For calibration, utilize the kit "Calibration Serum" Sclavo Code B35181702.

Traceability
The CK-NAC traceability is indicated in the calibration serum package insert.

SAMPLE
Sample types and storage
Serum and plasma with heparin or EDTA.

In serum / plasma	2 days	20-25 °C
	7 days	4-8 °C
	4 weeks	-20 °C

PREPARATION OF THE REAGENT
Slight variations in coloration among batches will not affect test results.
Procedure (monoreactive)
Add 1 volume of RB + 4 volumes RA, shake gently. The reagent thus prepared is ready to use.
Stability
3 weeks at 2 – 8 °C
2 days at 15 – 25 °C

Procedure (Bireactive)
The reagents are ready-to-use liquids. After opening, the reagents remain stable until the expiration date if kept in the conditions indicated in "Storage and stability."

Automation
The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD
The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

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Reaction conditions

Wavelength (primary):	340 nm
Temperature:	37°C
Reaction	Kinetic (Increasing Reaction)

Technique Monoreactive

Bring the reagents reaction temperature and operate away from direct light.

	U.M.	Blank	Calib. serum	Sample
Reagent (A+B)	µL	1000	1000	1000
Calib. serum	µL	-	40	-
Sample	µL	-	-	40
Water	µL	40	-	-

Mix well and after 3 minutes' wait carry out reading at 37°C.
Read sample and calibration serum absorbance subtracting absorbance of blank reagent, and complete reading within 3 minutes.

Technique – Bireactive

	U.M.	Blank	Calib. serum	Sample
Reagent A	µL	1000	1000	1000
Calib. serum	µL	-	50	-
Sample	µL	-	-	50
Water	µL	50	-	-
Mix well and incubate at 37°C for 3 min. and add				
Reagent B	µL	250	250	250

Mix well and after 2 minutes' wait carry out reading at 37°C.
Read sample and calibration serum absorbance for 3 minutes, subtracting absorbance of blank reagent.
The reaction volumes can be varied proportionally without altering the results.

Results:

Manual Method

Calculation of CK-NAC concentration:

$$\frac{\Delta \text{D.O.Sample}}{\Delta \text{D.O.Calibrator Serum CK-NAC}} \times \text{Calibrator Serum CK-NAC(U/L)} = \text{U/L CK-NAC}$$

Automation

The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically from the different instruments.

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L di CK-NAC
K-factor = 4127

REFERENCE RANGE

Adults		U.M.
Female	<145	U/L
Male	<171	U/L
Child		
Umbilical cord blood	175 - 402	U/L
Newborns	468 - 1200	U/L
≤ 5 days	195 - 700	U/L
< 6 months	41 - 330	U/L
> 6 months	24 - 229	U/L

Every laboratory must set its own normal-range values based on the population under study.

CHARACTERISTICS/PERFORMANCE

Linearity

Reaction is linear up to 1100U/L

Accuracy/Recovery

Commercial control sera were analyzed with the kit in question following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Mean (U/L)	DS	CV%	Recovery
Low	5	97.2	0.837	0.86	103.3%
High	5	706.8	5.119	0.72	103.2%

Interference

Interference	Limits
Asorbic Acid	**30 mg/dL**
Bilirubin	**40 mg/dL**
Triglycerides	**2000 mg/dL**
Hemoglobin	**200 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	mg/dL	159	3.18	2.00	20
High	mg/dL	508	3.69	0.73	20
Total precision (Within-lab precision)					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	mg/dL	49.5	1.05	2.12	20
High	mg/dL	228	2.31	1.01	20

Limits of sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is 7.0 U/L of CK-NAC in conditions established for this test.

Comparison between methods

The method was compared with a similar commercially available method.
The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-0.249
Slope	0.997
Correlation Coeff. (R)	0.99

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCES

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3. **NCCLS Document,** "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. **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.
5. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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