CK-NAC

Creatine Kinase Liquid Reagent

CE

In Vitro Diagnostic reagent for the quantitative determination of Creatine Kinase (CK-NAC) in serum and plasma. Store at 2-8°C .

REF: NAC-017B1 / NAC-017B2

Summary:

CK is a dimeric enzyme occurring in 4 different forms, a Mitochondrial Iso-enzyme and 3 Cytoplasmic Iso- enzymes. CK MM is a muscle enzyme, CK BB is a brain enzyme and CK MB is the heart enzyme. CK activity is elevated in many diseases including those involving skeletal muscle, heart, central nervous system and the thyroid. Most determinations of CK in the clinical laboratory are used for the early detection of Myocardial Infarction in which the enzyme is elevated within 3 to 8 hours after the attack.

Principle:

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

ATP + Glucose $\xrightarrow{Hexokinase}$ Glucose-6-Phosphate + ADP

Glucose-6-Phosphate + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ 6-phosphogluconate + NADPH + H^+

Composition:

R1: Imidazole Buffer pH 6.7 - 100 mmol/l, Glucose - 20 mmol/l, Magnesium Acetate - 10 mmol/l, EDTA - 2.0 mmol/l, ADP - 2.0 mmol/l, AMP - 5.0 mmol/l, NADP - 2.0 mmol/l,
HK - >2.5 U/ml, N-acetylcysteine - 20 mmol/l
R2: Creatine Phosphate - 30 mmol/l, G6P-DH - >1.5 U/ml, Diadenosine pentaphosphate - 10 µmol/l

Precautions:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.
Components Colour and Appearance:
Both Reagent: Clear colourless liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damaged to the bottle and/or cap, resulting in product leakage and/or contamination: do not use the reagent pack and contact your distributor .
Safety precautions:
Contains small quantities of Sodium Azide. Material Safety Data Sheet is available upon request.
Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

Instruments:

Instrument applications are available upon request.

Preparation:

Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label.
Monoreagent procedure: Mix 4 volumes of R1 with 1 volume of R2. Working reagent is stable 20 days at 2-8°C.
Bireagent procedure: Liquid reagent 1 and 2 are ready for use.

Samples:

Serum free of haemolysis is the preferred sample as plasma may produce unpredictable reaction rates.
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding sample handling. Serum should be separated from cells as rapidly as possible after collection.
Stability: up to 7 days if stored in a lightproof, tightly closed tube and maintained at 4°C

Equipment:

- General Chemistry Calibrator
- General Chemistry Control Level 1
- General Chemistry Control Level 2
- Photometer
- General Laboratory Equipment

Procedure:

1. Assay conditions:
Wavelength: ..... λ: 340 (334/365) nm
Cuvette: ....1 cm. light path
Temperature ..... .30°C (25°C or 37°C)

2.Monoreagent Procedure:

	Blank	Calibrator	Sample
Working Reagent (µL)	1000	1000	1000
Sample (µL)	-	-	-
Calibrator (µL)	-	40	-

Gently mix and Incubate at 25°C, 30°C or 37°C for 2 minutes.
Measure the change of Optical Density per minute (ΔOD/min) over the next 3 minutes.

3.Bireagent Procedure:

	Blank	Calibrator	Sample
R1(µL)	800	800	800
Sample (µL)	-	-	40
Calibrator (µL)	-	40	-

Gently mix and Incubate at 37°C for 5 minutes

R2(µL)	200	200	200

Gently mix and Incubate at 37°C for 2 minutes
Measure the change of Optical Density per minute (ΔOD/min) over the next 4 minutes

Enzyme Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Calculations:

$$\frac{\Delta OD\ Sample - \Delta OD\ Blank}{\Delta OD\ Calibrator - \Delta OD\ Blank} \times Concentration\ of\ Calibrator. = CK\ NAC\ Activity$$

(Conversion factor: Qty in µKat/l = Qty in U/l x 0.0167).

Quality control:

All clinical laboratories should establish an Internal Quality Control program. Check instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

Reference values:

The probability of myocardial injury is high when the following 3 conditions are met:

			U/L at 25°C	µkat/l at 25°C	U/L at 30°C	µkat/l at 30°C	U/L at 37°C	µkat/l at 37°C
1.	CK	Men	> 80	> 1.33	> 130	> 2.17	>190	> 3.17
		Women	> 70	> 1.17	> 110	> 1.83	> 167	> 2.87
2.	CK- MB		> 10	> 0.17	> 15	> 0.25	> 24	> 0.40
3.	CK-MB activity accounts for 6 – 25% of the total CK activity							

* Calculated values. Temperature conversion factors: 25°C to 30°C: 1.53. 25°C to 37°C: 2.38
Each laboratory should establish its own reference range. CK-MB results should always be reviewed with the patient's medical examination and history.

Performance characteristics:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.
Linearity:
Linear up to 1806 U/L (30 µkat/l).
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.
Interfering substances:
Bilirubin (mixed isomers): Less than 10% interference up to 400µmol/l Bilirubin
Haemolysis: Less than 10% interference up to 1.25 g/l Haemoglobin.
Lipemia: Less than 10% interference up to 5 g/l Intralipid.
Sensitivity:
The Lowest Detectable Level is estimated at 5 U/L (0.42 µkat/l).
Precision:

	Intra-assay			Inter-assay		
N=20	Mean (µmol/L)	SD	%CV	Mean (µmol/L)	SD	%CV
level 1	177	2.5	1.41	169	1.50	0.89
level 2	408	3.43	0.84	383	1.29	0.34

Method comparison:
Using 50 samples, a comparison, between this CK-NAC test (y) and another commercially available test (x), gave the following results:
y =0.997x + 5.765
r = 0.986
Sample range: 9 to 395 U/l

References:

1. Ann. Biol. Clin.40 (1982) 99. & Stein W. Med Weit. 1985, 36:572.
2. Stein W. Med Weit. 1985, 36:572 & Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54, 352-390 and 974-97
3. Szasz G, Busch EW. Third European Congress of Clinical Chemistry, Brighton, England, 3-8 June 1979 (abstract)

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