

CK-NAC Reagent - IFCC Single Vial

(Creatine Kinase, activated by N-Acetyl Cysteine)

PRODUCT SUMMARY

Stability	:	7 days at 2-8°C
Linear Range	:	Up to 1500 U/L
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of buffer.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of CK (ATP: Creatine N-phosphotransferase, EC 2.7.3.2) in human serum on both manual and automated systems.

CLINICAL SIGNIFICANCE

Creatine kinase (CK) is a dimeric enzyme composed of two types of monomer sub-units, M (Muscular) and B (Brain) which combine to form three distinct CK isoenzymes, CK-1 (BB), CK-2 (MB) and CK-3 (MM). The main proportion of total CK activity is found in the skeletal muscle and this is predominantly the CK-3 isoform. Other tissues with relatively high levels of CK include the myocardium, of which approximately 40% is the CK-2 isoform, gastrointestinal tract and brain where the CK-1 isoform predominates. Damage or disease to any of these tissues such as muscular dystrophy, myocardial infarction and acute cerebro vascular accident, will result in elevated blood levels of the enzyme.

METHODOLOGY^{1,2,3}

The CK-NAC, IFCC single vial reagent is based on the principles of the IFCC recommended procedure.

The series of reactions involved in the assay system is as follows:

- 1) Inactivated CK + NAC \longrightarrow Reactivated CK
- 2) Creatine Phosphate + Mg-ADP \xrightarrow{CK} ATP + Creatine
- 3) ATP + Glucose \xrightarrow{HK} ADP + G-6-P
- 4) G-6-P + NADPH⁺ $\xrightarrow{G6PDH}$ 6-PG + NADPH + H⁺
- 5) 2ADP $\xrightarrow{AK} //$ AMP + ATP
(Inhibited by P¹P⁵-diAP and AMP)

1. As CK in serum is rapidly inactivated, in order to ensure full catalytic activity, the CK molecule must be reactivated by a thiol compound. During the first stage, sample incubates with the thiol compound N-acetyl cysteine (NAC) which reactivates the CK molecule by rapidly reducing oxidised sulfhydryl compounds at the active site.
2. In the second stage the substrate creatine phosphate initiates a series of catalysed reactions. In the first of these reactions CK catalyses the formation of ATP from creatine phosphate and ADP.
3. ATP formed in 2 is used to form glucose-6-phosphate in a reaction catalysed by Hexokinase.
4. Glucose-6-phosphate produced in 3 is oxidised to 6-phosphogluconate and NADP is reduced to NADPH in a reaction catalysed by Glucose-6-phosphate dehydrogenase.
5. AMP and P¹P⁵-Di(adenosine-5'-)pentaphosphate (P¹P⁵-diAP) are added to inhibit adenylate kinase (myokinase) activity.

Abbreviations:

ADP	=	Adenosine-5'-diphosphate
ATP	=	Adenosine-5'-triphosphate
HK	=	Hexokinase
G-6-P	=	Glucose-6-phosphate
NADP ⁺	=	Nicotinamide Adenine Dinucleotide Phosphate
G-6-PDH	=	Glucose-6-phosphate dehydrogenase
6-PG	=	6-Phosphogluconate
NADPH	=	Reduced NADP
AMP	=	Adenosine-5'-monophosphate
AK	=	Adenylate Kinase
P ¹ P ⁵ -diAP	=	P ¹ P ⁵ -Di(adenosine-5'-)pentaphosphate

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use	REAG B	Reagent B
REAG A	Reagent A		Contains Mercury

REAGENT COMPOSITION

Active Ingredient	Concentration
AMP	5.25 mmol/L
NADP	2.2 mmol/L
P ¹ P ⁵ -diAP	10.5 µmol/L
EDTA	2.1 mmol/L
Mg ²⁺	11.6 mmol/L
ADP	2.1 mmol/L
D-Glucose	21 mmol/L
N-acetyl-L-cysteine	21 mmol/L
Hexokinase (Yeast)	>3,000 U/L
G-6-PDH (Leuconostoc)	>2,000 U/L
Imidazole Acetate	116 mmol/L
Creatine Phosphate	31.5 mmol/L
pH 6.75 ± 0.1 at 20°C.	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Contains Mercury. Dispose of according to local, state and federal regulations. For further information consult the CK-NAC Reagent - IFCC Single Vial Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

REAGENT PREPARATION

Reconstitute Reagent A with the volume of buffer, Reagent B, indicated on the vial label. Mix gently until dissolved.

STABILITY AND STORAGE

Prior to use:

When stored between 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Reconstituted Reagent:

When stored capped at 2-8°C, the reagent is stable for at least 7 days.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent Absorbance >0.6 AU at 340nm (1cm lightpath); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING¹

Serum: Use non-haemolysed serum.

Plasma: Avoid the use of plasma containing heparin, EDTA, citrate or fluoride.

Storage: CK is stable for 1 day at 4°C. Stability may vary somewhat for individual serum and is dependent upon the isoenzyme distribution and patient acid-base status.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	340 nm (334, 365nm)
Secondary Wavelength	405 nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 20
eg: Sample Vol	0.05 mL
Reagent Vol	1.0 mL

Delay/Lag	120 seconds
Read Time	3 minutes
Reagent Blank Limits (340 nm, 1cm lightpath)	Low 0.0 AU High 0.6 AU
Linearity (refer to Linearity section)	Up to 1500 U/L
Sensitivity (340 nm, 1cm lightpath)	0.30 ΔmA/min per U/L

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000}{6.3 \times \text{SV} \times \text{P}}$$

where: TV = Total reaction volume in mL
 SV = Sample volume in mL
 6.3 = millimolar absorption coefficient of NADH at 340nm
 (See note 4).
 P = Cuvette pathlength in cm.

Example: ΔAbs/min = 0.027
 Factor = 3333
 CK = 0.027 x 3333 = 90 U/L

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- If the change in absorbance is greater than 0.45/min repeat the assay with diluted serum. However, the volume fraction of serum in the CK reaction system is critical. Changes in the volume fraction, as will occur in sample predilution, does not produce stoichiometric changes in the reaction rate. If dilution is necessary 150 mmol/L of NaCl is recommended. At a dilution of 1:2 an apparent increase in CK of maximally 10% may be expected.² Alternatively, a CK free serum pool can be used for dilution. CK free serum can be produced by heating serum at 56°C for two hours.
- Valid results depend on accurately calibrated instruments, timing and temperature control.
- The millimolar absorption coefficient for NADH at 334 nm = 6.18 and at 365 nm = 3.40.
- Unit conversion: U/L x 16.67 x 10⁻³ μkat/L

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on an automated clinical chemistry analyser. The following results were obtained:
Haemoglobin: Avoid haemolysed specimens since red cells contain reaction intermediates such as ATP and G-6-P.⁴
Bilirubin: No interference from bilirubin up to 340 μmol/L (20 mg/dL).
Lipaemia: No interference from lipaemia, measured as triglycerides, up to 2.8 mmol/L (250 mg/dL).
- Young DS⁵ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁶

At 37°C	Males	≤200	(3.3 μkat/L)
	Females	≤180	(3.0 μkat/L)
At 30°C	Males	≤130	(2.1 μkat/L)
	Females	≤113	(1.9 μkat/L)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁷

*Calculated results using a temperature conversion of 0.625 for 30°C. Thermo does not recommend the routine use of temperature conversion factors.

PERFORMANCE DATA

The following data was obtained using the CK-NAC IFCC Single Vial Reagent on an automated clinical chemistry analyser.

IMPRECISION

Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.⁸

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	134	393
SD (U/L)	4.3	5.2
CV (%)	3.2	1.3

Between Day:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	134	393
SD (U/L)	5.2	16.3
CV (%)	3.9	4.1

ACCURACY

Comparison studies were carried out using a similar commercially available CK-NAC reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	60
Range of sample results	17 - 749 U/L
Mean of reference method results	148 U/L
Mean of CK-NAC results	147 U/L
Slope	1.01
Intercept	-1.15 U/L
Correlation coefficient	0.999

LINEARITY

When run as recommended, the assay is linear up to 1500 U/L.


SENSITIVITY

When run as recommended the sensitivity of this assay is 0.30 ΔmA/min per U/L.

REFERENCES

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 Fisher Diagnostics
 a division of Fisher Scientific Company, LLC
 a subsidiary of Thermo Fisher Scientific Inc.
 Middletown, VA 22645-1905 USA
 Phone: 800-528-0494
 540-869-3200
 Fax: 540-869-8132

 MDCI Ltd.
 Arundel House
 1 Liverpool Gardens
 Worthing, West Sussex BN11 1SL UK



REF

Reorder Information

Catalogue No.	REAG A	REAG B
TR14110	20 x 10 mL	1 x 200 mL
TR14115	20 x 20 mL	2 x 200 mL
TR14103	10 x 50 mL	1 x 500 mL
TL14101 (I Lab 600)	20 x 20 mL	1 x 400 mL