CK-NAC REAGENT
(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 or plasma
Method:	Kinetic
Reagent Preparation:	Add specified volume of distilled or deionized water.

SYMBOLS IN PRODUCT LABELLING

EC REP Authorized Representative
IVD For in vitro diagnostic use
LOT Batch code/Lot number
REF Catalogue number
Consult instructions for use
Temperature Limitation
Use by/Expiration Date
CAUTION. CONSULT INSTRUCTIONS FOR USE.
Manufactured by
Exclamation Mark

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 or 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 isoenymes, 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
The CK-NAC reagent is based on the method of Oliver[1] modified by Rosalki[2] and Szasz.[3] The series of reactions involved in the assay system is as follows:

1) Inactivated CK + NAC ⟶ Reactivated CK

2) Creatine Phosphate + Mg-ADP $\xrightarrow{CK}$ ATP + Creatine

3) ATP + Glucose $\xrightarrow{HK}$ ADP + G-6-P

4) G-6-P + $NADP^+$ $\xrightarrow{G6PDH}$ 6-PG + NADPH + H^+

5) 2ADP $\xrightarrow{AK}$ // ⟶ AMP + ATP
(Inhibited by AP5A 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 sulfydryl 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 (AP5A) 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
AP5A	= P_1P_5 -Di(adenosine-5'-)pentaphosphate

REAGENT COMPOSITION

Active Ingredient	Concentration
Bis / Tris Buffer	100 mmol/L
Creatine Phosphate	31.5 mmol/L
AMP	5.3 mmol/L
NADP	2.2 mmol/L
EDTA	2.1 mmol/L
AP5A	10.3 µmol/L
Mg^{2+}	10.5 mmol/L
ADP	2.7 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

pH 6.80 ± 0.1 at 20°C.

Hazard Symbol: Exclamation Mark
Signal Word: Warning

Hazard Statements
H319 Causes serious eye irritation
Precautionary Statements - Prevention
Wash face, hands and any exposed skin thoroughly after handling
Wear eye/face protection
Precautionary Statements - Response
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
80.824% of the mixture consists of ingredient(s) of unknown toxicity
Other information
May be harmful if swallowed
Causes mild skin irritation
Refer to the product Safety Data Sheet for additional information.

The Packaging for This Product Contains Dry Natural Rubber. For further information consult the CK-NAC reagent Material Safety Data Sheet.

REAGENT PREPARATION
Reconstitute the contents of each vial with the volume of distilled or deionized water stated on the vial label. Mix gently until dissolved.

STABILITY AND STORAGE
Prior to Reconstitution:
When stored at 2-8°C reagent is stable until the expiration date stated on the vial and kit box label.

Reconstituted Reagent:
When stored capped at 2-8°C, the reagent is stable for at least 7 days. It is recommended that when the reagent is not in use for prolonged periods of time (eg: overnight) that the reagent be capped and stored at 2-8°C.

Indications of Reagent Deterioration:
- Turbidity;
- Reagent Absorbance >0.5 AU at 340nm (1cm lightpath); and /or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING[4]

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
- A clinical chemistry analyser capable of maintaining constant temperature (30°/37°C) and measuring absorbance at 340 nm (335, 365 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, 365 nm)
Secondary Wavelength	405 nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 20
eg: Sample Vol	15 µL
Reagent Vol	300 µL

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Delay/Lag	120 seconds	
Read Time	3 minutes	
Reagent Blank	Low	0.0 AU
(1cm lightpath, 340nm)	High	0.5 AU
Linearity	1500 U/L	
(refer to Linearity section)		
Sensitivity	0.30 ΔmA/min per U/L	
(1cm lightpath, 340nm)		

Calculations

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

Activity in U/L = ΔAbs/min x Factor

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

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

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. 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.[5] 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.
3. Valid results depend on accurately calibrated instruments, timing and temperature control.
4. The millimolar absorption coefficient for NADH at 334 nm = 6.18 and at 365 nm = 3.40.
5. 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

1. Studies to determine the level of interference from haemoglobin, bilirubin (free and conjugated), lipaemia, ascorbic acid and glucose were carried out. The following results were obtained:

Haemoglobin:	Avoid haemolysed specimens since red cells contain reaction intermediates such as ATP and G-6-P.
Free Bilirubin:	No interference from free bilirubin up to 500µmol/L (29mg/dL).
Conjugated Bilirubin:	No interference from conjugated bilirubin up to 500µmol/L (29mg/dL).
Lipaemia:	No interference from lipaemia, measured as triglycerides, up to 6mmol/L (531mg/dL).
Ascorbic Acid:	No interference from ascorbic acid up to 2.5 mmol/L.
Glucose:	No interference from glucose up to 25 mmol/L (450 mg/dL).

2. The temperature of the fluid used to reconstitute lyophilised control serum has been reported to affect catalytic activity.[4]
3. For a more comprehensive review of factors affecting urea assays refer to the publication by Young.[6]

EXPECTED VALUES[7]

At 37°C	Males	≤175	(2.9 µkat/L)
	Females	≤140	(2.3 µkat/L)
At 30°C	Males	≤105	(1.8 µkat/L)
	Females	≤ 80	(1.3 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.[8]

PERFORMANCE DATA

The following data was obtained using the CK-NAC reagent on an automated clinical chemistry analyser.

IMPRECISION:

Imprecision was evaluated using two levels of commercial control.

Within Run:

	LEVEL I	LEVEL II
Number of data points	20	20
Mean (U/L)	134	456
SD (U/L)	2.34	4.06
CV (%)	1.75	0.89

Between Run:

	LEVEL I	LEVEL II
Number of data points	20	20
Mean (U/L)	132	428
SD (U/L)	3.51	15.24
CV (%)	2.66	3.56

ACCURACY:

Comparison studies were carried out using another 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	25 - 561 U/L
Mean of reference method results	141 U/L
Mean of CK-NAC results	140 U/L
Slope	0.997
Intercept	2.5 U/L
Correlation coefficient	0.9995

LINEARITY:

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

SENSITIVITY:

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

REFERENCES

1. Oliver IT. Biochem J. 1955; 61:116-22.
2. Rosalki SB. J. Lab. Clin. Med. 1967; 69: 696-705.
3. Szasz G., Waldentsrom J., Gruber W. Clin Chem. 1979; 3:446-52.
4. Hørder M., Elser R.C., Gerhardt W., et al. Journal of the IFCC 1989; 1:130-8.
5. Strömme JH., Theodorsen L., Hørder M., et al. Scand. J. Clin. Lab. Invest. 1976; 36: 711-23.
6. Young DS. Effects of drugs on clinical laboratory tests. Third edition, 1990; 3: 120-22.
7. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994.
8. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

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REF

Reorder Information

Catalogue No.	Configuration
TR14010	20 x 10 mL