TRUEchemie CK NAC Test Kit (IFCC)

for the quantitative determination of Creatine Kinase (CK) in human serum

INTENDED USE

The TRUEchemie CK NAC Test Kit (IFCC) is used for the quantitative determination of Creatine Kinase (CK) in human serum

INTRODUCTION

Creatine Kinase (CK) plays an important role in the energy-storing mechanism of tissue by catalyzing the reversible reaction between creatine and ATP to form creatine phosphate- and ADP. CK is distributed in various organs; the highest activities (in decreasing order) are skeletal muscle, heart, and brain. Thus, determination of CK is an aid in diagnosing muscular dystrophy and other diseases of the skeletal muscles, myocardial infarction, hypothyroidism, renal diseases, and/or dysfunction.

The early procedure for determining CK was based on the rate of ATP formation. A modified method was described by Nielson by adding a sulfhydryl compound and AMP to assure maximum CK activity and inhibit adenylate kinase activity. Optimized conditions for measuring CK were published by Szasz in 1976 as well as by the Scandinavian committee on enzyme. The above procedure was modified again in 1979 to include EDTA. The present reagent is a modification of the above revision.

PRINCIPLE

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

$$\text{ATP + D-Glucose} \xrightarrow{\text{HK}} \text{Glucose-6-Phosphate + ADP}$$

$$\text{Glucose-6- phosphate + NAD} \xrightarrow{\text{G-6-PDH}} \text{6- Phosphogluconate + NADH + H}^{+}$$

CK catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. The ATP and glucose are converted to ADP and glucose-6-phosphate by hexokinase (HK). Glucose-6-phosphate dehydrogenase (G-6-PDH) oxidizes at the D-glucose-6-phosphate and reduces the nicotinamide adenine dinucleotide (NAD). The rate of NADH formation, measured at 340 nm, is directly proportional to serum CK activity.

PACK SIZE

Kit size	**2 x 25 ml**
Cat. no.	**ADX342**
Kit contents	
1) CK NAC Reagent (R1)	2 x 20 ml
2) CK NAC Reagent (R2)	2 x 5 ml

REAGENTS COMPOSITION

CK NAC Reagent (R1) and (R2) come in separate containers, and both reagents are clear, colorless liquid in ready to use format. After combining CK NAC Reagent (R1) & CK NAC Reagent (R2) the reagent composition:

Working reagent composition

D-Glucose	: 20 mmol/L
Magnesium	: 10 mmol/L
Adenosine-5'-monophosphate (AMP)	: 50 mmol/L
N-Acetylcysteine (NAC)	: 20 mmol/L
Creatine phosphate	: 30 mmol/L
Adenosine-5'-diphosphate (ADP)	: 2 mmol/L
Oxidized nicotinamide adenine dinucleotide phosphate	: 2 mmol/L
Glucose-6-phosphate dehydrogenase	: 3,000 IU/L
Hexokinase	: 3,000 IU/L
EDTA	: 2 mmol/L
Buffer	: 100 mmol/L

REAGENT PREPARATION

The working reagent is prepared by mixing 4 volumes of R1 with 1 volume of R2 in a disposable container or mixing 0.800 ml of R1 with 0.200 ml of R2 in test tube.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The disposal of the residues has to be done as per local legal regulations.

REAGENT STORAGE & STABILITY

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN COLLECTION AND STORAGE

Collect whole blood by non-traumatic venipuncture and allow to clot. Centrifuge and remove serum immediately. Serum is reportedly stable for 4 hours at room temperature, 8 - 12 hours at 4°C, and 2-3 days when frozen.

Hemolyzed specimens should not be used because of side reactions that may occur due to adenylate kinase, adenosine triphosphate, and glucose-6-phosphate dehydrogenase liberated from red cells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 340 nm

TEST PROCEDURE

Wavelength : 340 nm
Temperature : 37°C
Prewarm the reagent to reaction temperature.

	Blank (µl)	**Sample (µl)**
Distilled Water	1000	--
CK NAC Reagent (R1)	--	800
CK NAC Reagent (R2)	--	200
Sample	--	40

Reading & Calculations

Blank the Photometer with D.I Water.
Mix, read the absorbance after 1 min and start the stopwatch. Read again the absorbance after 1, 2 and 3 minutes
Calculations:
ΔE = Initial absorbance - Absorbance at 1st, 2nd, 3rd min.
Calculations determine the ΔE/min. for every reading and find the mean value.
(Avg ΔE/min.) x 5450 = IU/L of CK

SI UNITS: To convert to SI Units (nKat/L) multiply IU/L by 16.67.

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

Male : 30 - 200 IU/L
Female : 29 - 168 IU/L
It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

Sensitivity: 3.0 IU/L
Linearity: Up to 2000 IU/L under the described assay conditions. If the concentration is greater than linearity (2000 IU/L), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=10)	**Mean (U/L)**	**SD (U/L)**	**CV (%)**
Control Level - 1	124.4	0.3	0.3
Control Level - 2	458.4	0.3	0.1
Inter-assay precision run to run (n=12)	**Mean (U/L)**	**SD (U/L)**	**CV (%)**
Control Level - 1	128.1	0.5	0.4
Control Level - 2	465.2	0.8	0.2

The reagent was tested for 12 days, using two different CK NAC concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie CK NAC reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.999 and the regression equation is y=0.995x+0.509. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Certain drugs and medications may affect the activity of CK.

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEMS PARAMETERS

Mode	:	Kinetic
Factor	:	5450
Wave length	:	340 nm
Units	:	IU/L
Flow cell temp.	:	37 °C
Blank	:	Distilled water
Reagent volume	:	800 µL (R 1) + 200 µL (R2)
Sample volume	:	40 µL
Lag time	:	60 Sec. (1 min.)
Read time	:	180 Sec. (3 min.)
Low normal	:	29
High normal	:	200
Sensitivity	:	3
Linearity	:	2000
Reaction slope	:	Increasing

REFERENCES

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Index of Symbols

Consult instructions for use	REF Catalogue number	Caution
IVD *In vitro* diagnostic medical device	LOT Batch code	Non-sterile
2°C 8°C Temperature limit 2-8 °C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry
Do not use if package is damaged	Keep away from sunlight	

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