Liquick Cor-CREA ENZYMATIC

DIAGNOSTIC KIT FOR DETERMINATION OF CREATININE CONCENTRATION

Kit name	**Cat. No**
Liquick Cor-CREA ENZYMATIC mini	2-227
Liquick Cor-CREA ENZYMATIC 30	2-257
Liquick Cor-CREA ENZYMATIC 60	2-267

INTRODUCTION

Creatinine is a product of creatine nonenzymatic dehydration in skeletal muscle. The amount of creatinine generated and excreted by kidney is proportional to muscle mass and usually is higher in men than women. Daily creatinine generation remains fairly constant, with the exception of crushing injury or degenerative diseases that cause massive damage to muscle. Creatinine blood and urine level depends on glomelural filtration so creatinine clearance is excellent index of renal function.

METHOD PRINCIPLE

Enzymatic, colorimetric method.

creatinine + H_2O $\xrightarrow{\text{creatininase}}$ creatine

creatine + H_2O $\xrightarrow{\text{creatinase}}$ sarcosine + urea

sarcosine + O_2 + H_2O $\xrightarrow{\text{sarcosine oxidase}}$ glycine +HCHO + H_2O_2

$2H_2O_2$ + ESPMT + 4-AA $\xrightarrow{\text{peroxidase}}$ quinoneimine dye + $4H_2O$

The colour intensity measured at 546 nm is proportional to the creatinine concentration.

REAGENTS

Package

	Liquick Cor-CREA ENZYMATIC mini	**Liquick Cor-CREA ENZYMATIC 30**	**Liquick Cor-CREA ENZYMATIC 60**
1-CREA ENZYMATIC	2 x 18 ml	3 x 30 ml	3 x 60 ml
2-CREA ENZYMATIC	1 x 12 ml	1 x 30 ml	1 x 60 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

Components and concentrations

1-CREA ENZYMATIC	
Good's buffer	≤ 5%
creatinase	≤ 5%
N-ethyl-N-(3-sulfopropyl)-3-methylaniline (ESPMT)	≤ 5%
sarcosine oxidase	≤ 0.01%
ascorbate oxidase	≤ 1%
detergents, stabilizers and preservatives	
2-CREA ENZYMATIC	
Good's buffer	≤ 5%
creatininase	≤ 1%
peroxidase	≤ 5%
4-ammoantipyrine (4-AA)	≤ 0.01%
stabilizers and preservatives	

Warnings and notes

- Product for in vitro diagnostic use only.
- 2-CREA ENZYMATIC contains sodium azide (< 0.1%) as a preservative. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 546 nm (550 nm);
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum and urine.

Urine preparation: before analysis urine sample should be diluted with 0,9% NaCl 2-10 times. Multiply the result by dilution factor.

Serum can be stored up to 1 day at 2-8ºC. For longer storage samples should be frozen at -20ºC.

Urine can be stored up to 1 day at 20-25ºC, 4 days at 2-8ºC. For longer storage samples should be frozen at -20ºC.

Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

NOTE:

1-CREA ENZYMATIC volume, and respectively the sample and 2-CREA ENZYMATIC volumes should be adjusted to measuring photometer capacity.

Manual procedure

wavelength	546 nm (550 nm)
temperature	37°C
cuvette	1 cm

Pipette into the cuvettes:

	standard (S)	test (T)	blank (B)
1-CREA ENZYMATIC	900 µl	900 µl	900 µl

Bring up to the temperature of determination. Then add:

standard	30 µl	-	-
sample	-	30 µl	-
distilled water	-	-	30 µl

Mix well and incubate for 5 minutes at 37 ºC. Read the absorbance A_1 of standard samples A(S) and test sample A(T) against reagent blank (B). Then add:

2-CREA ENZYMATIC	300 µl	300 µl	300 µl

Mix well. Incubate for 5 minutes at 37 ºC. Read the absorbance A_2 of standard samples A(S) and test sample A(T) against reagent blank (B). Calculate ΔA (A_2-A_1) for the test and standard.

Calculation

$\Delta A(T) = (A_2\text{-}A_1)T \times K$

$\Delta A(S) = (A_2\text{-}A_1)S \times K$

$$\text{Creatinine concentration [mg/dl]} = \frac{\Delta A(T)}{\Delta A(S)} \times \text{standard / calibrator concentration}$$

K = (sample volume + R1 volume) / (sample volume + R1 volume + R2 volume)

K = 0.756

REFERENCE VALUES [2, 3]

serum / plasma	**mg/dl**	**µmol/l**
newborns	0.3 – 1.0	26.5 – 88.4
infants	0.2 – 0.4	17.7 – 35.4
children	0.2 – 0.8	17.7 – 70.7
female	0.5 – 1.0	44.2 – 88.4
male	0.7 – 1.2	61.9 – 106.1
urine (morning)	**mg/dl**	**mmol/l**
female	29 – 226	2.56 – 20.0
male	40 – 278	3.54 – 24.6

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) for determination in serum or CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) or LEVEL 2 (Cat. No 5-162) for determination in urine with each batch of samples.

For the calibration of manual assay the CREATININE STANDARD 2 (Cat. No 5-123), CREATININE STANDARD 5 (Cat. No 5-124).

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176), LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 4 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.08 mg/dl (7.07 µmol/l).

- **Linearity:** up to 24 mg/dl (2122 µmol/l).

- **Specificity / Interferences**
  Haemoglobin up to 5 g/dl, ascorbic acid up to 62 mg/l, bilirubin up to 20 mg/dl, triglycerides up to 1000 mg/dl and creatine up to 20 mg/dl both at serum and urine do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	1.23	0.02	1.23
level 2	5.63	0.04	0.67

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	1.17	0.04	3.63
level 2	5.51	0.30	5.42

- **Method comparison**

A comparison between creatinine values determined at Biolis 24i Premium (y) and at Prestige 24i (x) using 31 samples gave following results:

$y = 0.9661 x + 0.0226$ mg/dl;

$R = 0.9903$ (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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Date of issue: 07. 2015.

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07/15/07/15