LIQUIZYME

CREATININE

(ENZYMATIC METHOD)

BEACON

Code	Product Name	Pack Size
LS017A	Liquizyme Creatinine (Enzymatic)	40 ml
LS017B	Liquizyme Creatinine (Enzymatic)	80 ml
LS017C	Liquizyme Creatinine (Enzymatic)	160 ml

Intended Use

Diagnostic reagent for quantitative *in vitro* determination of Creatinine in human serum, plasma and urine.

Clinical Significance

Creatnine is a waste product formed in muscle from the high energy storage compound, creatine phosphate. The amount of creatinine produced is fairly constant (unlike Urea) and is primarily a function of muscle mass. It is not greatly affected by diet, age, sex or exercise. Creatinine is removed from plasma by glomerular filtration and then excreted in urine without any appreciable resorption by the tubules.

Creatinine is used to assess renal function, however, serum creatinine levels do not start to rise until renal function has decreased by at least 50%.

Principle

In the first reaction, creatinase and sarcosine oxidase are used in the enzymatic hydrolysis of endogenous creatine to produce hydrogen peroxide, that is eliminated by catalase.

Creatininase and 4-aminoantipyrine are added, and only the creatine generated from creatinine by creatininase is hydrolysed sequentially by creatinase and sarcosine oxidase to produce hydrogen peroxide. This newly formed hydrogen peroxide is measured in a coupled reaction catalysed by peroxidase, with N-ethyl-N-sulphopropyl-m-toluidine (ESPMT) as a chromogen.

The absorbance of the produced complex at 546 nm is proportional to the creatinine concentration in the sample.

Reaction

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatininase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{Sarcosine Oxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$2H_2O_2 + \text{4-AA}^{+1} + \text{TOOS}^{-2} \xrightarrow{\text{Peroxidase}} \text{Quinone} + 4H_2O$$

1 : 4-Aminoantipyrine
2 : N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine

Creatinine concentration can be obtained by measuring quinone pigment photometrically.

Reagent Composition

Reagent 1 : Creatinine R1

Buffer pH 8.0	:	25 mmol/L
Creatinase	:	>20 KU/l
Sarcosine oxidase	:	>5 KU/l
Ascorbate oxidase	:	<3 KU/l
Catalase	:	>80 KU/l
ESPMT	:	0.5 mmol/L

Reagent 2 : Creatinine R2

Buffer pH- 7.6	:	>25 mmol/L
Creatininase	:	>250 KU
Peroxidase	:	>20 KU/l
4-aminoantipyrine	:	>2 mmol/L

Reagent 3 : Creatinine Standard : 2 mg/dl

Reagent Preparation

Reagents R1 and R2 are liquid, ready to use.

Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Stability

In Serum / Plasma :

7 days	:	at 4 – 25°C
at least 3 months	:	at - 20°C

In Urine :

2 days	:	at 20 – 25°C
6 days	:	at 4 – 8°C
3 months	:	at - 20°C

Materials Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pippetes or Micropioettes & Tips
- Colorimeter or Bio-Chemistry Analyzer.

Specimen Collection And Handling

Use serum, plasma, urine. It is recommended to follow NCCLS procedures (or similar standardized conditions).

For the determination in urine use 24 hours specimen. It is important to exactly measure the volume of collected urine. Dilute urine samples in 1+19 ratio with distilled water and multiply results by 20.

Discard contaminated specimens.

Calibration

Calibration with Creatinine standard provided in the kit is recommended.

Quality Control

It's recommended to run normal and abnormal control sera to validate reagent performance.

Assay Procedure For Serum

Wavelength : 546 nm

Addition Sequence	Reagent Blank	Standard	Sample
Reagent 1	450 µl	450 µl	450 µl
Standard	-	50 µl	-
Sample	-	-	50 µl
Mix and incubate 5 min. At 37°C. Then add			
Reagent 2	150 µl	150 µl	150 µl

Mix and incubate for 5 min at 37° C. Measure the absorbance of test and standard against the reagent blank.

Calculation

$$\text{Creatinine Concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 2$$

Assay Procedure For Urine

Wavelength : 546 nm
Cuvette : 1 cm

Addition Sequence	Reagent Blank	Standard	Sample
Reagent 1	450 µl	450 µl	450 µl
Standard	-	10 µl	-
Sample	-	-	10 µl
Mix and incubate 5 min. At 37°C. Then add			
Reagent 2	150 µl	150 µl	150 µl

Mix and incubate for 5 min at 37° C. Measure the absorbance of test and standard against the reagent blank.

Calculation

$$\text{Creatinine Concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 2$$

Unit Conversion

mg/dl x 88.4 = µmol/l

Expected Values

Serum Male : 0.6 - 1.1 mg/dl
Female : 0.5 - 0.8 mg/dl
Urine 1070 - 2150 mg/dl (24 hrs. accumulated urine)
769 - 1200 mg/dl (24 hrs. accumulated urine)

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 0.042 mg/dl
Linearity : 40 mg/dl (Serum) & 200 mg/dl (Urine)
Measuring range : 0.042 – 40 mg/dl (Serum) & 0.042 – 200 mg/dl (Urine)

Intra-assay precision Within run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	5.50	0.08	1.48
Sample 2	1.61	0.06	3.77

Inter-assay precision Run to run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	0.62	0.020	3.24

Comparison

A comparison between Liquizyme Creatinine (Enzymatic Method) (y) and a commercially available test (x) using 20 samples gave following results :

y = 0.909 x + 0.145
r = 0.999

Interferences

Following substances do not interfere :
hemoglobin up to 5 g/l, bilirubin up to 30 mg/dl, triglycerides up to 1000 mg/dl.

Warning And Precautions

For *in vitro* diagnostic use. To be handles by entitled and professionally educated person. Reagents of the kit are not classified like dangerous but Reagent R2 contains less than 0.1% sodium azide - classified as toxic and dangerous substance for the environment.

Waste Management

Please refer to local legal requirements.

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	End point
Wavelength 1 (nm)	546
Sample Volume (µl)	50 (Serum) / 10 (Urine)
Reagent Volume (µl)	450 + 150
Incubation time (min.)	5 + 5
Incubation temp. (°C)	37
Normal Low (mg/dl)	0.6 (Serum), 1070 (Urine)
Normal High (mg/dl)	1.1 (Serum), 2150 (Urine)
Linearity Low (mg/dl)	0.042 (Serum), 0.042 (Urine)
Linearity High (mg/dl)	40 (Serum),200 (Urine)
Standard Concentration	2 mg/dl
Blank with	Reagent
Unit	mg/dl

References

1. Kaplan, L. A., Pesce, A. J.: Clinical Chemistry, Mosby Ed. (1996)

Symbols Used On Labels

REF Catalogue Number
Manufacturer
See Instruction for Use
LOT Lot Number
CONT Content
Storage Temperature
Expiry Date
IVD In Vitro Diagnostics

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JAS-ANZ BSCIC ISO 9001 : 2015 BSCIC ISO 13485:2016