CREATININE (Enzymatic)

(ENZYMATIC METHOD)

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Ref. CC3-CEN.024, 2x20 ml
CC3-CEN.24U, 2x40 ml
CC3-CEN.24V, 3x80 ml

INTENDED USE

Creatinine Reagent is an enzymatic assay intended for in vitro quantitative detection of Creatinine in human serum on automated clinical chemistry analyzers.

DIAGNOSTIC SIGNIFICANCE

Creatinine is one of the decomposition products of muscle metabolism, and then filtered and excreted by kidney in urine. High concentration creatinine is in every urine samples of health human beings. It is commonly used to be a marker of undiluted urine in case of samples being replaced for avoiding drug test. A rise of creatinine level in blood also indicates renal function deficiency by reflecting the glomerular filtration rate decreased. Be careful that the test result of creatinine concentration detection is easy to be affected by gender, age, weight or even daily intake.

PRINCIPLE

The detecting method adopted in this kit is enzymatic colorimetric determination which is developed for overcoming the chromogenic interferences in Jaffe method.

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

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

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

$$2H_2O_2 + \text{4-AAP} + \text{EHSPT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

*4-AAP = 4-Aminoantipyrine

*EHSPT = N-Ethyl-N-(2-Hydroxy-3-Sulfopropyl)-m-Toluidine

The addition of quinoneimine dye results in an increase in absorbance at 546nm which is directly proportional to the amount of creatinine in the sample.

PRESENTATION

	No of bottle / Vial		
Store all reagents at 2 to 8°C	2x20 ml	2x40 ml	3x80 ml
• 1 - Creatinine	2x15 ml	2x30 ml	3x60 ml
• 2 - Creatinine	2x5 ml	2x10 ml	3x20 ml
• Creatinine Calibrator (value stated on vial label)	1	1	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Good's Buffer	100mmol/L
• Sarcosine oxidase	>18KU/L
• Creatine amidinohydrolase	>40KU/L
• Ascorbic acid oxidase	>8KU/L
• Sodium n-ethyl-n -(2-hydroxy-3-sulfopropyl) - 3-methylaniline dehydrate	1.2mmol/L
• Creatinine amidohydrolase	>270KU/L
• Peroxidase	>25KU/L
• 4-AAP	2.5mmol/L
• Creatinine	≥ 0.05g/L

PRECAUTIONS

1. The results are only for clinical reference. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment reactions;
2. The reagent is only for in vitro diagnosis use, not oral administration.
3. To detect the same sample, the result shall be different from different manufacturers.
4. All samples and reaction wastes should be treated as infectious sources and operators should take necessary protective measures.
5. The reagent contains preservatives to avoid touching the skin and mucosal tissue. If you are accidentally spilled, please rinse it with clean water immediately and go to the hospital if necessary.
6. The reagent and sample volume shall be adjusted in proportion to the requirements of different biochemistry analyzers.

PREPARATION OF WORKING REAGENT

The reagents are liquid and ready to use.

REAGENT STORAGE AND STABILITY

The reagents shall be stored at 2-8℃. Do not freeze. The reagents are stable when stored as instructed until the expiration date on the label. The on-board stability shall be 30 days.
CRE calibrator shall be stored at 2-8℃. The calibrator is liquid and stable when stored properly until the expiration date on the label.

SPECIMEN COLLECTION

Fresh serum without hemolysis.

CALIBRATION

CRE Calibrator is liquid and ready to use. Two-point calibration is adopted. Calibrator which offered is recommended. If you use other manufacturer's calibrator, please verify by yourself. Please do re-calibration if the reagent lot number change, quality control drift, instrument maintenance or important parts replacement.

QUALITY CONTROL

Quality control should be carried out before samples are tested every day to ensure the stability of the test system. Using commercially available controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

The results of quality control should be within the allowable range. If the results deviate from the scope, please take the following steps to find out the reasons:

1. Check whether the parameter setting and the light source are correct.
2. Check the cleanliness of the colorimetric cup and sampling needle.
3. Check whether the water is contaminated or not. Bacterial growth can lead to incorrect results.
4. Check the reaction temperature.
5. Check the validity of the reagent.

TEST PROCEDURE

Sample Volume	3 uL
Reagent 1 (R1)	150 uL
Mix Sample and R1 well and incubate for 5 minutes at 37°C, read absorbance A1. Then, add:	
Reagent 2 (R2)	50 uL
Mix well and incubate for another 5 minutes and read the absorbance A2, calculate △A=A2-A1.	
Main wavelength	546 nm
Sub wavelength	700 nm
Test method	Endpoint
Reaction direction	Increase

TEST RESULTS

$$\text{Creatinine (mg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. calibrator}} \times \text{Calibrator Con.}$$

LIMITATIONS FOR INTERFERENCE

If the concentration of interfering substances in the sample satisfies the following requirements, the test results will not be affected.
Ascorbic acid (≤0.5g/L)
Bilirubin (≤0.4g/L)
Hemoglobin (≤5g/L)
Triglyceride (≤10g/L)

NORMAL VALUES

Male: 0.6 – 1.39 mg/dl
Female: 0.5 – 1.2mg/dl
It is recommended that each laboratory should establish its own reference range.

EXPLANATION OF TESTING RESULT

Professionals are responsible for the audit of inspection results. The test results will be affected by age, sex, weight and so on. Usually, the results are considered normal within the reference range. If the results out of the range, they should be re-determined for confirmation. And if they are clearly beyond the reference range or even beyond the reference range after confirmation, the target content in serum is considered abnormal.

PERFORMANCE CHARACTERISTICS

1. Appearance:
   R1: Yellowish clarifying liquid.
   R2: Colorless to yellowish clarifying liquid.
   CRE Calibrator: colorless clarifying liquid.
2. Linearity: 17 mg/dl
3. Precision: CV≤5%
4. Inter-batch deviation: R≤10%
5. Accuracy: The measured value shall be fallen within the range of quality control target value.
6. Blank absorbance: The absorbance value of the reagent is ≤0.050 at the wavelength of 546nm and the optical path of 1 cm.

REFERENCES

1. Saito zhenghang et al. Clinical chemical analysis II. Tokyo chemistry Tongren, 531968
2. Anyuan Zhengshan et al. Reagents for clinical test instruments, 8 (5), 11831985