CheKine™ Mirco Creatinine (Cr) Content Assay Kit

Cat #: KTB1002 Size: 48 T/48 S 96 T/96 S

	Mirco Creatinine (Cr) Content Assay Kit		
REF	**Cat #:** KTB1002	LOT	**Lot #:** Refer to product label
	Applicable sample: Serum, Plasma, Urine, and other liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Creatinine is a metabolic byproduct of muscle metabolism in the human body and is primarily excreted through glomerular filtration by the kidneys. The sources of creatinine in the blood include both endogenous and exogenous components. Serum creatinine is almost entirely filtered through the glomeruli into the primary urine and is not reabsorbed by the renal tubules. The daily production of endogenous creatinine remains almost constant, and when the intake of exogenous creatinine is strictly controlled, the serum creatinine concentration reaches a stable value. Therefore, measuring the serum creatinine concentration can reflect the glomerular filtration function.CheKine™ Mirco Creatinine (Cr) Content Assay Kit provides a simple, convenient, and rapid method for detecting creatinine content, suitable for liquid samples such as serum, plasma, urine, etc. The principle of this assay is based on the hydrolysis of creatinine to creatine by creatinine amidohydrolase. Creatine is then converted to sarcosine and urea by creatine amidinohydrolase. Sarcosine, one of the products, is oxidized by sarcosine oxidase to generate sarcosine, hydrogen peroxide, and formaldehyde. Finally, the hydrogen peroxide reacts with chromogenic substrates 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine under the catalysis of peroxidase to produce a red quinimine compound. Since quinimine has a maximum absorption peak at a wavelength of 546 nm, within a certain concentration range, the change in absorbance at 546 nm is directly proportional to the creatinine content in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	60 μL	120 μL	-20℃, protected from light
Reagent Ⅲ	12 mL	24 mL	4℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅴ	18 μL	36 μL	-20℃, protected from light
Reagent Ⅵ	3.6 mL	7.2 mL	4℃, protected from light

ReagentⅦ	1 mL	1 mL	4℃
Standard	1 mL	1 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 546 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, ice maker, low-temperature centrifuge
- Deionized water, physiological saline

Reagent Preparation

Working Reagent Ⅰ: Prepare immediately before use: Add a small amount of Reagent III (no more than 0.5 mL) to Reagent I and Reagent II, and mix thoroughly by pipetting to dissolve. Use a pipette to completely transfer the dissolved reagent into Reagent III (this step can be repeated multiple times until no residual powder remains), and mix thoroughly. Keep on ice and protect from light throughout the experiment; store at 4℃, protected from light to 2 weeks.

Working Reagent Ⅱ: Prepare immediately before use: Add a small amount of Reagent VI (no more than 0.5 mL) to Reagent IV and Reagent V, and mix thoroughly by pipetting to dissolve. Use a pipette to completely transfer the dissolved reagent into Reagent VI (this step can be repeated multiple times until no residual powder remains), and mix thoroughly. Keep on ice and protect from light throughout the experiment; store at 4℃, protected from light to 2 weeks.

Standard: Prepare immediately before use: Dilute 20 μL of Standard with 980 μL of deionized water to achieve a concentration of 200 μg/mL. Mix thoroughly and use immediately.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Serum (Plasma): Direct detection.
2. Urine: It is recommended to dilute with physiological saline 2-10 times before testing, and multiply the result by the dilution factor.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 546 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Note: Before measurement, add 186 μL of Reagent Ⅶ to 1-2 wells and measure the absorbance A_0 at 546 nm. Before calculating, subtract A_0 from both A_1 and A_2.

Reagent	Test Well (μL)	Standard Well (μL)	Blank Well (μL)
Sample	6	0	0
Standard	0	6	0
Ddeionized water	0	0	6
Working Reagent Ⅰ	180	180	180
Mix thoroughly and incubate at 37℃ for 5 min. Measure the absorbance A_1 at 546 nm, recording the values as A_{1Test}, $A_{1Standard}$, and A_{1Blank}, respectively. Continue by adding the following reagents:			

Working Reagent II	60	60	60

Mix thoroughly and incubate at 37℃ for 5 min. Measure the absorbance A_2 at 546 nm, recording the values as A_{2Test}, $A_{2Standard}$, and A_{2Blank}, respectively.

Note: Blank well and standard well only need to be measured once. Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre experiment. If A_{2Test} is less than 0.05, the sample volume can be appropriately increased, and the calculation formula should be adjusted accordingly; If A_{2Test} is greater than 0.8, the sample can be further diluted with physiological saline, and the calculated result is multiplied by the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculate $\Delta A_{Test}=A_{2Test}-K\times A_{1Test}$, $\Delta A_{Standard}=A_{2Standard}-K\times A_{1Standard}$, $\Delta A_{Blank}=A_{2Blank}-K\times A_{1Blank}$.

Where (K) is the dilution factor, calculated as:$K=(V_{Sample}+V_1)\div(V_{Sample}+V_1+V_2)=186\div246=0.756$

Creatinine content (mmol/L)$=(\Delta A_{Test}-\Delta A_{Blank})\div(\Delta A_{Standard}-\Delta A_{Blank})\times C_{Standard}\times$Dilution factor

$=1{,}768\times(\Delta A_{Test}-\Delta A_{Blank})\div(\Delta A_{Standard}-\Delta A_{Blank})\times$Dilution factor

V_1: Reaction volume after adding sample to Working Reagent I: 186 μL; V_2: Final reaction volume after adding sample to both Working Reagent I and Working Reagent II: 246 μL; $C_{Standard}$: Standard concentration=200 μg/mL=1,768 μmol/L

Typical Data

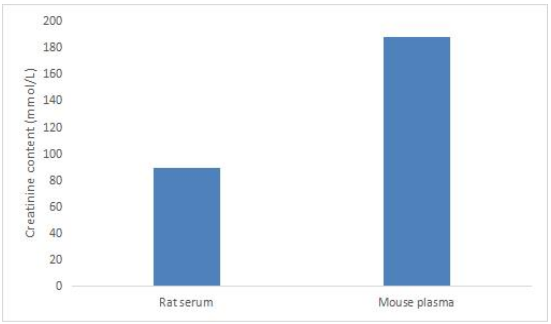

Figure 1. Determination the creatinine content in rat serum and mouse plasma by this assay kit

\Recommended Products

Catalog No.	Product Name
KTB1111	CheKine™ Micro D-lactate Dehydrogenase (D-LDH) Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.