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
	Detection range: 20-8,000 μmol/L		**Sensitivity:** 20 μmol/L
	Applicable sample: Animal Tissues, Serum, Plasma, Urine, and other liquid samples		
	Storage: Stored at 4℃ for 12 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 animal tissues, 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 F-DAOS 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. This kit contains anti-interference components such as ascorbic acid oxidase and EMse447, which can effectively solve the interference of endogenous and other drugs in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	12 mL	24 mL	4℃, protected from light
Reagent Ⅱ	4 mL	8 mL	4℃, protected from light
Standard	100 μL	100 μL	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

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

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

1. Animal tissues: Weigh about 0.1 g tissues and add 1 mL physiological saline. Homogenize on ice. Centrifuge at 6,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Serum (Plasma): Direct detection.
3. Urine: It is recommended to dilute with deionized water 100 times before testing, and multiply the result by the dilution factor.

Note: If the protein concentration is calculated, the protein needs to be extracted with deionized water for determination. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

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	4	0	0
Standard	0	4	0
Ddeionized water	0	0	4
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:			
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. Calculate $\Delta A_{Test}=A_{2Test}-A_{1Test}$, $\Delta A_{Standard}=A_{2Standard}-A_{1Standard}$, $\Delta A_{Blank}=A_{2Blank}-A_{1Blank}$, $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$, $\Delta\Delta A_{Standard}=\Delta A_{Standard}-\Delta A_{Blank}$.			

Note: Blank well and standard well only need to be measured 1-2 times. Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre experiment. If the reagent becomes turbid or the A_{Blank} is greater than 0.1, it cannot be used and should be discarded. If $\Delta\Delta A_{Test}$ is less than 0.005, increase the sample quantity appropriately. If the concentration of CREA in the sample is greater than 8,000 μmol/L, the sample can be further diluted with physiological saline, and the calculated result can be 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.

1. Calculation of CREA concentration in samples:

(1) Calculated by fresh weight of samples

CREA (nmol/g fresh weight)=$C_{Standard}$×△ΔA_{Test}÷△Δ$A_{Standard}$×V_{Sample}÷(W×V_{Sample}÷$V_{Sample\ Total}$)**×n=442×△△A_{Test}÷△△A_{Test}÷W×n**

(2) Calculated by protein concentration

CREA (nmol/mg prot)=$C_{Standard}$×△ΔATest÷△ΔAStandard×VSample÷(Cpr×V_{Sample})**×n=442×△△A_{Test}÷△△A_{Test}÷Cpr×n**

(3) Calculation by liquid volume:

CREA (μmol/L)=$C_{Standard}$×△ΔATest÷△ΔAStandard**×n=442×△△A_{Test}÷△△A_{Test}×n**

Where: $C_{Standard}$: 442 μmol/L=442 nmol/mL; Cpr: Sample protein concentration, mg/mL; V_{Sample}: Sample volume added to the reaction system, 0.004 mL; W: Sample weight, g; $V_{Sample\ Total}$: Sample Preparation of added physiological saline volume, 1 mL; n: Sample dilution factor.

Typical Data

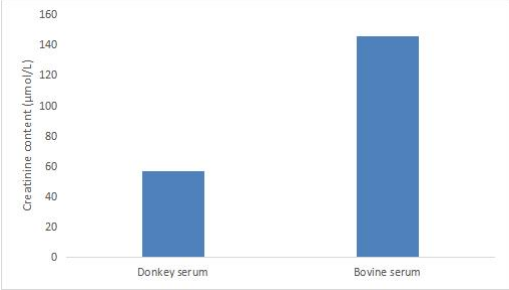

Figure 1. Determination the creatinine content in donkey serum and bovine serum by this assay kit

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.