CheKine™ Micro Creatine Content Assay Kit

Cat #: KTB1003 Size: 48 T/48 S 96 T/96 S

	Micro Creatine Content Assay Kit		
REF	**Cat #:** KTB1003	LOT	**Lot #:** Refer to product label
	Detection range: 20-600 μg/mL		**Sensitivity:** 20 μg/mL
	Applicable sample: Animal Tissues, Serum (Plasma)		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Creatine is a nitrogen-containing organic acid primarily stored in the muscles and brain of vertebrates, where it participates in energy metabolism (facilitating rapid ATP generation via phosphocreatine). The levels of creatine in animals are influenced by factors such as diet, physical activity, breed, age, and health status. CheKine™ Micro Creatine Content Assay Kit provides a simple, convenient, and rapid approach for measuring creatine levels, suitable for animal tissue and serum (plasma) samples. The assay is based on the reaction of creatine with diacetyl-α-naphthol under alkaline conditions, forming a red-colored product with an absorption peak at 520 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	7.5 mL	15 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	0.1 mL	0.2 mL	4℃, protected from light
Reagent Ⅳ	25 mL	50 mL	4℃
Reagent Ⅴ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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 520 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube, funnel, filter paper
- Water bath, cryogenic centrifuge, ice maker

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• Deionized water
• Homogenizer (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Working ReagentⅡ: Prepared before use. Add 25 mL deionized water for 48 T and 50 mL deionized water for 96 T, dissolve by heating in a 60℃ water bath, and filter through filter paper. Unused reagent can be stored at 4℃ in the dark for up to 3 days. If crystals form, redissolve by heating in a 60℃ water bath.
Working ReagentⅢ: Prepared before use. Add 20 μL ReagentⅢ to 20 mL deionized water and mix thoroughly. Prepare fresh for each use. Unused ReagentⅢ should be stored at 4℃ in the dark.
Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Working Reagent V: Prepared before use. Add 20 mL Reagent IV to one vial of Reagent V and dissolve completely, then add 2 mL Working Reagent III and mix thoroughly. Use on the same day of preparation.
Note: Reagent II is moderately toxic, and Reagent III and Reagent V have irritating odors. It is recommended to perform experiments in a fume hood.
Standard: Prepared before use. Add 1 mL deionized water and fully dissolve to 10 mg/mL Creatine Standard Solution. The remaining reagent can also be stored at 4℃ for 1 month. Use the 10 mg/mL standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μg/mL)
Std.1	48 μL 10 mg/mL Standard	952	480
Std.2	40 μL 10 mg/mL Standard	960	400
Std.3	32 μL 10 mg/mL Standard	968	320
Std.4	24 μL 10 mg/mL Standard	976	240
Std.5	16 μL 10 mg/mL Standard	984	160
Std.6	8 μL 10 mg/mL Standard	992	80
Std.7	4 μL 10 mg/mL Standard	996	40
Blank	0	1,000	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.
1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL deionized water and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Serum (Plasma): Test directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 nm, visible spectrophotometer was returned to zero with deionized water.
2. Adjust the water bath to 30℃.

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3. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Standard Tube (μL)
Deionized Water	100	100
Reagent Ⅰ	100	100
Sample	100	0
Standard	0	100
Working Reagent Ⅱ	100	100
Mix thoroughly, centrifuge at 10,000 g for 10 min at 4℃, and transfer the supernatant to a new 1.5 mL tube.		
Supernatant	40	40
Working Reagent Ⅴ	200	200
Mix well, keep still at room temperature for 5 min, then incubate in a 30℃ water bath for 30 min.		
Deionized Water	200	200

4. After mixing, pipette 200 μL of the solution into a 96-well plate or micro cuvette. Read the absorbance at 520 nm and document the values as A_{Test}, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{BlankI}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank and standard curve tubes need only be run 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.05, the sample volume can be appropriately increased. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get x (μg/mL).

2. Calculation of the creatine content:

(1) Calculated based on fresh weight of the sample:

Creatine (μg/g fresh weight)=$x\times V_{Total\ Sample}\div W\times F$**=x÷W×F**

(2) Calculated by volume of sample

Creatine (μg/mL)**=x×F**

$V_{Total\ Sample}$: Volume of deionized water added for extraction, 1 mL; W: sample weight; F: dilution factor.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

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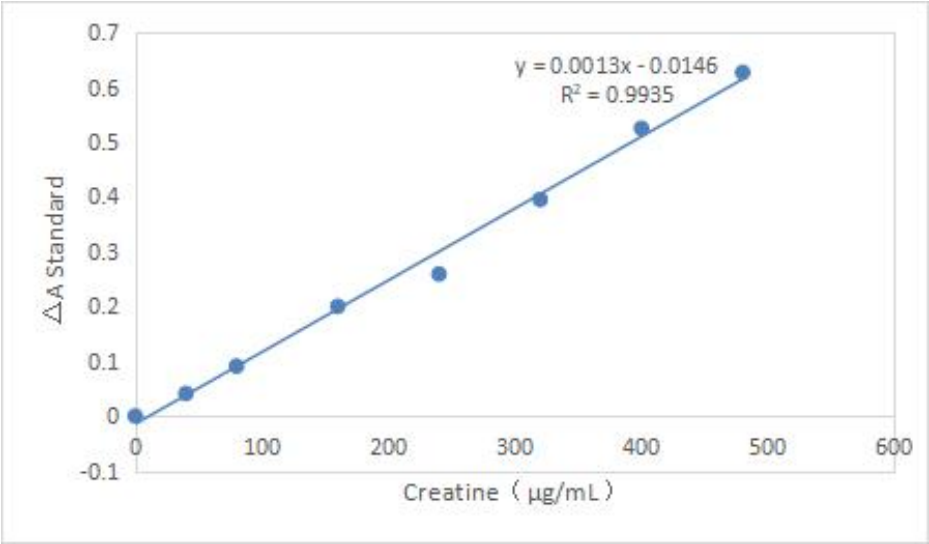

Figure 1. Creatine standard curve

Recommended Products

Catalog No.	Product Name
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1041	CheKine™ Micro Hydrogen Peroxide (H2O2) 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.

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