Creatine Content Assay kit

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC4925

Size: 100T/48S

Components:

Extracting solution I：Liquid 60 mL×1. Storage at 2-8℃.

Extracting solution II：Liquid 10 mL×1. Storage at 2-8℃.

Reagent I: Powder ×1. Storage at 2-8℃. Add 0.33mL of ethanol to dissolve the reagent before use, the reagent can be stored at -20℃ for 2 weeks, the reagent can not be reused if it turns dark brown.

Reagent I dilution: Liquid 5 mL×1. Storage at 2-8℃.

Reagent I working solution: before use, according to the sample volume according to the ratio of reagent 1: reagent 1 dilution = 40μL: 560μL (total of 600μL, about 15T) to be prepared, ready for use, used up on the same day;

Reagent II: Liquid 2 mL×1. Storage at 2-8℃.

Standard solution: Powder ×1, 1 mg of creatine monohydrate. Before use, add 1 mL of distilled water to fully dissolve, i.e. 1 mg/mL Creatine monohydrate standard stock solution. Store unused reagents at 2-8°C for one month.

Product Description:

Creatine is a nitrogen-containing compound, which is naturally found in vertebrates, and can assist in energy supply for muscle and nerve cells. Creatine can be synthesized by three amino acids, arginine, glycine and methionine, which can be synthesized by human body or taken from food. About 95% of creatine is found in skeletal muscle, mainly in the form of phosphocreatine. As a supplement, creatine can enhance the performance of the muscles by increasing the muscle quality. Creatine is also widely studied as a therapeutic drug for neuromuscular diseases, which may help to protect the nerves and improve the biological function of cells.

Creatine can react with diacetyl-α-naphthol in alkaline condition to form a red product with an absorption peak at 530 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, low tempareture centrifuge, transferpettor, micro glass cuvette/96 well plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation (the sample size can be adjusted appropriately, and the specific proportion can be referred to the literature):

1. Tissue:

Accordance ratio weight(g): Extract solution I(mL)=1: 5~10. (Suggested 0.1g tissue with 1mL Extract

solution I). Homogenate on ice bath. 12000 g centrifuge for 10 min at 4℃. Add 0.15mL Extract solution II slowly to 0.8 mL supernatant. **Blend slowly until no bubbles.** Then 12000 g centrifuge for 10 min at 4℃. Supernatant is for test.

2. Cells:

Accordance ratio cell amount (10^6): Extract solution I(mL)=5~10:1. (Suggested 5 million cells with 1mL Extract solution I). Breaking cells (300W, work time 3s, interval 7s for 3 min) by ultrasonic on ice bath. 12000 g centrifuge for 10 min at 4℃. Add 0.15 mL Extract solution II to 0.8mL supernatant slowly. **Blend slowly until no bubbles**. 12000 g centrifuge for 10 min at 4℃. Supernatant is used for test.

3. Serum (plasma) **sample:**

Add 1 mL Extract solution I to 100 μL serum(plasma). 12000 g centrifuge for 10 min at 4℃. Add 0.15 mL Extract solution II to 0.8 mL supernatant slowly. **Blend slowly until no bubbles.** Centrifuge for 10 min at 12000 g. Supernatant is used for test.

II. Determination procedure:

1. Preheat the Spectrophotometer/Microplate Reader for 30 minutes, adjust the wavelength to 530 nm, the Spectrophotometer set zero with distilled water.
2. Preparation of standard solution: dilute 1 mg/mL creatine monohydrate standard stock solution with distilled water to 200、160、130、100、50、25、12.5 mg/mL standard solution for use.
3. Add reagents with the following list:

Reagent（μL）	Test tube (T)	Control tube (C)	Blank tube (B)	Standard (S)
Sample	20	20	-	
Distilled water	-	20	20	
Standard solution	-	-	-	20
Reagent I	40	40	40	40
Reagent II	20	-	20	20
Reaction at room temperature without light for 10 min.				
Distilled water	120	120	120	120
Mix well, the absorbance at 530 nm is determined. They are respectively recorded as A_T, A_B and A_S. $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$.				

Note: Blank tubes and standard curves only need 1-2 times.

III. Calculation:

1. Standard curve drawing: take creatine monohydrate standard solution concentration as abscissa (x, mg/mL), take ΔA standard as ordinate (y) to draw standard curve, and get linear regression equation y= kx + b. bring ΔA_T into equation to get x (mg/mL).

2. Calculation formula

(1) Calculated according to protein concentration

Creatine content (μg/mg prot) = $x \times V_S \div (V_S \times Cpr) \times 0.879 = x \div Cpr \times 0.879$

(2) Calculated by sample quality

Creatine content (μg/g mass) = $x\times(V_{SE}+V_{E2})\div(W\times V_{SE}\div V_{E1})\times0.879=1.044\times x\div W$

(3) Calculated by the number of bacteria or cells

Creatine content (μg/10^4 cells) = $x\times(V_{ST}+V_{E2})\div$(cell number × $V_{ST}\div V_{E1}$)×0.879= 1.044×x÷cell number

(4) Calculated according to the volume of serum

Creatine content (μg/mL)= $x\times(V_{ST}+V_{E2})\div[V_L\times V_{ST}\div(V_{E1}+V_L)]\times0.879=11.482\times x$

V_S: volume of sample added, 100 μL = 0.1 mL; V_{ST}: volume of supernatant when extracting by Extracting solution I, 0.8 mL; Cpr: concentration of sample protein, mg/mL; W: mass of sample, g; 5: number of cells: 5×10^6; V_{E1}: volume of Extracting solution I, 1 mL; V_{E2}: volume of Extracting solution II, 0.15 mL; V_L: volume of liquid sample, 0.1 mL; 0.879: conversion coefficient, relative molecular weight of creatine monohydrate is 149.15, relative molecular weight of anhydrous creatine is 131.13, 0.879=131.13÷149.15.

Note:

1. After color development, please complete the test within 10 minutes.
2. The extract contains a protein precipitant and the extracted supernatant cannot be used for protein concentration determination. If you want to calculate the creatine content by protein concentration, you need to take another sample, i.e., take the same mass of tissue, the same number of bacteria or cells, and homogenize it with 1.1875 mL PBS (saline); take the same volume of serum (plasma), and homogenize it with 1.206 mL PBS (saline) (which is equivalent to the supernatant of the final sample of the extraction step), and then use the BCA method to determine the protein concentration.
3. If the absorbance value is lower or higher than the linear range, the sample size can be increased or diluted before the determination.
4. Reagent I and Reagent II are irritant to human body. Please take appropriate protective measures. For your safety and health, please wear lab clothes and latex gloves.

Experimental examples:

1. Take 0.1 g of rabbit kidney and add 1 mL of Extracting solution I for homogenate grinding and centrifugation. Take 0.8 mL of supernatant and add 0.15 mL Extracting solution II. After centrifugation, the supernatant is diluted twice, then operate according to the determination steps. After determination with 96 well plate, calculate: y=0.0062x-0.0143, $\Delta A_T=A_T-A_B=0.128-0.064=0.064$， x=12.629. The content is calculated according to the sample mass.

The content of creatine (μg/g mass) = 1.044 × x÷W× 2(dilution ratio) = 131.85 μg/g mass.

2. Take 100 μL of bovine serum, add 1 mL of Extracting solution I, take 0.8 mL of supernatant and add 0.15 mL of Extracting solution II, the centrifugal supernatant, and then operate according to the determination steps. After determination, calculate: y=0.0062x-0.0143, $\Delta A_T=A_T-A_B==0.271-0.07=0.201$， x=34.726. The content is calculated according to the volume of liquid。

The content of creatine (μg/mL) = 11.482×x= 398.7 μg/mL serum.