Creatine Content Assay kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC4925

Size: 100T/48S

Components:

Extracting solution I：Liquid 60 mL×1. Storage at 4℃.

Extracting solution II：Liquid 10 mL×1. Storage at 4℃.

Reagent I: Liquid 5 mL×1. Storage at 4℃.

Reagent II: Liquid 2 mL×2. Storage at 4℃.

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.

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. Preparation of bacteria and cell samples: according to the cell number (10^4): the volume of Extracting solution I (mL) is 500~1000:1 (it is recommended to add 1 mL of Extracting solution I to 5 million cells), ice bath ultrasonic wave is used to crush cells (power 300 W, ultrasonic 3 seconds, interval 9 seconds, total time 5 min); centrifugation at 4℃, 12000 g for 10 min, take 0.8 mL of supernatant, and then add 0.15 mL of Extracting solution II, After centrifugation at 4℃ and 12000 g for 10 min, the supernatant is taken for determination.

2. Preparation of tissue samples: according to the ratio of mass (g): the volume of Extracting solution I (mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Extracting solution I), add Extracting solution I, homogenize in ice bath, centrifuge at 4℃, 12000g for 10 min, take 0.8 mL of supernatant, and then add 0.15 mL of Extracting solution II, centrifuge at 4℃, 12000g for 10 min, take supernatant for testing.

3. Serum (plasma): take 100 μL of serum(plasma) and add 1 mL of Extracting solution I, centrifuge at 4℃, 12000g for 10 min, take 0.8 mL of supernatant, then add 0.15 mL of Extracting solution II, centrifuge at 4℃, 12000g for 10 min, and then take the supernatant for testing.

II. Determination procedure:

1. Preheat the Spectrophotometer/Microplate Reader for 30 minutes, adjust the wavelength to 530 nm, 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: the Blank tube only needs 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) = $x \times (V_{SE}+V_{E2}) \div (W \times V_{SE} \div V_{E1}) \times 0.879 = 1.044 \times x \div W$

(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)= $x \times (V_{ST}+V_{E2}) \div [V_L \times V_{ST} \div (V_{E1}+V_L)] \times 0.879 = 11.482 \times x$

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 supernatant can not be used for the determination of protein concentration. If you want to calculate creatine content with protein concentration, you need to take another tissue or serum (plasma), that is, take the same mass (volume) of tissue (serum (plasma)) with 1.1875 mL PBS (normal saline) homogenate (equivalent to the final sample supernatant of the extraction step), and use BCA method to determine 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) = 131.84 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.