Creatine Content (Enzymic Method) Assay kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: NA0211

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: Powder ×2. Storage at -20℃. Before use, add 550 μL of distilled water to each tube and dissolve it completely. The unused reagents is divided and then stored at -20℃.

Reagent II: Powder ×2. Storage at -20℃. Before use, add 150 μL of distilled water to each tube and dissolve it completely. The unused reagents is divided and then stored at -20℃.

Reagent III: Powder ×2. Storage at -20℃. Before use, add 0.5 mL of distilled water to each tube （100T/48S） and dissolve it completely. For the convenience of storage, give one more. The unused reagents is divided and then stored at -20℃.

Reagent IVA: Liquid 10 mL×1. Storage at 4℃.

Reagent IVB: Liquid 10 mL×1. Storage at 4℃. Before use, according to the amount required by the experiment, the mixture shall be fully mixed according to the ratio of Reagent IVA: Reagent IVB = 1:1, and prepare when the solution will be used.

Standard: 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. Before use, 20 μL of 1 mg/mL standard solution and 80 μL of distilled water are mixed to prepare a standard solution of 200 μg/mL for use and preparation.

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 be converted into glycine, formaldehyde and hydrogen peroxide by creatine enzyme coupled with sarcosine oxidase. Peroxidase catalyzes hydrogen peroxide to oxidize 4-aminoantipyrine coupled phenol to form colored compounds with characteristic absorption peak at 505 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, ultrasonic crusher.

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 505 nm, set zero with distilled water.
2. 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	20	20	20	20
Mix well and react for 10 min at 37℃ (mammalian) or 25℃ (other species).				
Reagent II	2	2	2	2
Reagent III	2	2	2	2
Reagent IV	160	160	160	160
Mix well, color for 30 min at 37℃ (mammalian) or 25℃ (other species). The absorbance at 505 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. Calculation formula

(1) Calculated according to protein concentration

Creatine content (μg/mg prot) = $C_S \times V_S \times \Delta A_T \div \Delta A_S \div (V_S \times Cpr) \times 0.879 = 175.8 \times \Delta A_T \div \Delta A_S \div Cpr \times 0.879$

(2) Calculated by sample quality

Creatine content (μg/g mass) = $C_S \times \Delta A_T \div \Delta A_S \times (V_{ST} + V_{E2}) \div (W \times V_{ST} \div V_{E1}) \times 0.879 = 208.76 \times \Delta A_T \div \Delta A_S \div W$

(3) Calculated by the number of bacteria or cells

Creatine content (μg/10^4 cells) ==$C_S \times \Delta A_T \div \Delta A_S \times (V_{ST} + V_{E2}) \div (\text{cell number} \times V_{ST} \div V_{E1}) \times 0.879$
$= 208.76 \times \Delta A_T \div \Delta A_S \div \text{cell number}$

(4) Calculated according to the volume of serum

Creatine content (μg/mL)= $C_S \times \Delta A_T \div \Delta A_S \times (V_{ST} + V_{E2}) \div [V_L \times V_{ST} \div (V_{E1} + V_L)] \times 0.879 = 2296.39 \times \Delta A_T \div \Delta A_S$

C_S: standard tube concentration, 200 μg/mL; V_S: add volume of sample, 20 μL = 0.02 mL; V_{ST}: volume of supernatant during extraction, 0.8 mL; V_{E1}: add volume of Extracting solution, 1 mL; V_{E2}: add volume of Extracting solution II, 0.15 mL; W: sample mass, g; Cpr: sample protein concentration, mg/mL; cell number: 10^4; 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 exceeds the absorbance value of the standard tube, it is recommended to dilute the sample with distilled water before determination. If the absorbance value is too small, it is recommended to increase the sample size before determination.

Experimental examples:

1. Take 0.1g rabbit kidney and add 1 mL of Extracting solution I for homogenate grinding and centrifugation. Take 0.8 mL supernatant and add 0.15 mL of Extracting solution II. After centrifugation, operate according to the determination steps. After determination with 96 well plate, calculate $\Delta A = \Delta A_T - A_C = 0.108 - 0.074 = 0.034$, $\Delta A_S = A_S - A_B = 0.806 - 0.053 = 0.753$. The content is calculated according to the sample mass.

Creatine content (μg/g mass) = $208.76 \times \Delta A_T \div \Delta A_S \div W$ = 94.26 μg/g mass.

3. 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: $\Delta A_T=A_T-A_C=0.122-0.062=0.06$, $\Delta A_T= A_S-A_B= 0.806 - 0.053 = 0.753$. The content is calculated according to the volume of liquid.

The content of creatine (μg/mL) = $2296.39\times\Delta A_T\div\Delta A_S=282.98$ μg/mL serum.