Creatinine Content Assay Kit (Creatine Oxidase Method)

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

Operation Equipment: Spectrophotometer

Cat No: BC4910

Size: 50T/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 -20℃. Before use, add 3.6 mL of distilled water to each tube and dissolve it completely. The unused reagents can be divided and then stored at -20℃ in 4 weeks.

Reagent II: Powder ×1. Storage at -20℃. Before use, add 3.6 mL of distilled water to each tube and dissolve it completely. The unused reagents can be divided and then stored at -20℃ in 4 weeks.

Reagent III: Powder ×1. Storage at -20℃. Before use, add 1 mL of distilled water to each tube and dissolve it completely. The unused reagents can be divided and then stored at -20℃ in 4 weeks.

Reagent III working solution: according to the required amount of the test, according to the ratio of Reagent III: distilled water = 1:9, fully mix and use immediately.

Reagent IV: Liquid 1 mL×1. Storage at 2-8℃. Before use, add 1 mL of distilled water and dissolve it completely. The unused reagents is divided and then stored at -20℃ in 4 weeks.

Reagent V: Powder ×1. Storage at -20℃. Before use, add 7.2 mL of distilled water to each tube and dissolve it completely. The unused reagents is divided and then stored at -20℃ in 4 weeks.

Reagent VIA: Liquid 10 mL ×1. Storage at 2-8℃.

Reagent VIB: Liquid 10 mL ×1. Storage at 2-8℃. Before use, according to the amount required by the experiment, the mixture shall be fully mixed according to the ratio of Reagent VIA: Reagent VIB = 1:1, and prepare when the solution will be used.

Standard: Powder ×1, 10 mg of creatinine. Before use, add 10 mL of distilled water to fully dissolve, i.e. 10 mg/mL standard solution. Before use, 20 μL of 10 mg/mL standard solution and 980 μL of distilled water are mixed to prepare a standard solution of 200 μg/mL, prepare when the solution will be used.

Product Description:

Creatinine (Cre), the chemical formula is $C_4H_7N_3O$, is a product of muscle metabolism in the human body, which is mainly excreted by glomerular filtration. Serum creatinine comes from both exogenous and endogenous sources. Exogenous creatinine is the product of meat metabolism in the body; endogenous creatinine is the product of muscle metabolism in the body.

The creatinine in the sample is hydrolyzed to creatine under the catalysis of creatinine enzyme. Under the catalysis of creatinase, creatine hydrolyzes to creatine and urea. Sarcosine is oxidized to

hydrogen peroxide under the catalysis of sarcosine oxidase. Peroxidase catalyzed the oxidation of 4-aminoantipyrine

coupling phenol by hydrogen peroxide to form colored compounds with characteristic absorption peak at 505nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, low tempareture centrifuge, transferpettor, 1 mL glass cuvette, mortar/homogenizer/ ultrasonic crusher, 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 Extracting solution I to 5 million cells), ice bath ultrasonic wave is used to crush cells (power 300W, 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 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)	Blank tube (B)	Standard (S)
Sample	60	-	-
Distilled water	-	60	-
Standard solution	-	-	60
Reagent I	60	60	60
Reagent II	60	60	60
Reagent III working solution	15	15	15

Reagent IV	15	15	15
Mix well and react for 10 min at 37℃ (mammalian) or 25℃ (other species).			
Reagent V	120	120	120

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com

Reagent VI	270	270	270
Mix well, color for 30 min at 37℃ (mammalian) or 25℃ (other species).			
Distilled water	400	400	400
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

Creatinine content (μg/mg prot) = $C_S \times \Delta A_T \div \Delta A_S \times V_S \div (V_S \times Cpr) = 200 \times \Delta A_T \div \Delta A_S \div Cpr$

(2) Calculated by sample quality

Creatinine content (μg/g mass)= $C_S \times \Delta A_T \div \Delta A_S \times (V_U+V_{E2}) \div (W \times V_U \div V_{E1}) = 237.5 \times \Delta A_T \div \Delta A_S \div W$

(3) Calculated by the number of bacteria or cells

Creatinine content (μg/10^4 cells)= $C_S \times \Delta A_T \div \Delta A_S \times (V_U+V_{E2}) \div (\text{cell number} \times V_S \div V_{E1}) = 237.5 \times \Delta A_T \div \Delta A_S \div$ cell number

(4) Calculated according to the volume of serum

Creatinine content (μg/mL)= $C_S \times \Delta A_T \div \Delta A_S \times (V_U+V_{E2}) \div (V_{E1}+V_L)] = 2612.5 \times \Delta A_T \div \Delta A_S$

C_S: standard tube concentration, 200 μg/mL; V_S: add sample volume, 60 μL = 0.06 mL; V_U: volume of supernatant during extraction, 0.8 mL; V_{E1}: add the volume of Extracting solution I, 1 mL; V_{E2}: add the volume of Extracting solution II, 0.15 mL; W: sample mass, g; Cpr: sample protein concentration, mg/mL; cell number: 10^4; V_L: the volume of liquid sample, 0.1 mL.

Note:

1. The supernatant can not be used for the determination of protein concentration. If you want to calculate creatinine 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.
2. If the test tube absorbance value exceeds that of the standard tube, it is recommended to dilute the sample before measuring. If the absorbance value is too small, it is recommended to increase the sample before determination.

Experimental examples:

1. Take 0.1 g of rabbit muscle and add 1 mL of Extracting solution I for homogenization and centrifugation. Take 0.8 mL of supernatant and add 0.15 mL Extracting solution II. After centrifugation, operate according to the determination steps. After determination with 96 well plate, calculate $\Delta A_T = A_T - A_B = 0.133 - 0.032 = 0.101$, $\Delta A_S = A_S - A_B = 0.563 - 0.032 = 0.531$. The content is calculated according to the sample mass.

The content of creatinine (μg/g mass) = 237.5 × $\Delta A_T \div \Delta A_S \div W$ = 436.04μg/g mass.

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com