Creatinine (Cr) Content Assay Kit (SOX, Micro Method)

Cat. No.: C1515992 | Pack size: 48T / 96T | Storage: 2-8 °C, protected from light

Overview

Creatinine (CRE) is a metabolite produced by muscle metabolism, which is mainly excreted via glomerular filtration. Under normal physiological conditions, the internal creatinine level remains relatively stable. Serum creatinine concentration serves as one of the key indicators for evaluating glomerular filtration function. This assay kit adopts an enzymatic method: creatinine is specifically catalyzed by creatininase to generate creatine. Subsequently, creatine is sequentially acted upon by creatinase and sarcosine oxidase to produce hydrogen peroxide. Hydrogen peroxide reacts with chromogenic reagent to form purple-colored products with a maximum absorption peak at 546 nm, and the creatinine content is calculated accordingly.

Kit Contents & Storage

Item	Value
Storage Conditions	Store at 2-8 °C, protected from light
Shipped In	Wet ice
Stability and Storage	Store at 2-8 °C for 3 months. Store in the dark.

Cat. No.	Component	Appearance	48T	96T	Storage	Notes
C1515992A	Reagent I	Liquid	10 mL	18 mL	2-8 °C. Store in the dark.	
C1515992B	Reagent II	Liquid	3.5 mL	6 mL	2-8 °C. Store in the dark.	
C1515992C	Standard tube	Solid	2 mg	2 mg	2-8 °C. Store in the dark.	1. Centrifuge at 8000 g, 4 °C for 2 min before use to collect powder at the tube bottom. 2. Add 1 mL distilled water to prepare 2 mg/mL standard stock solution. 3. Further dilute 40 times (20 µL stock solution + 780 µL water) to obtain 0.05 mg/mL (442 µmol/L) working creatinine standard solution.

Experimental Instruments

Mortar/homogenizer, analytical balance, ice box/ice maker, tabletop centrifuge, adjustable pipette, water bath incubator/drying oven/cell incubator/metal bath, 96-well plate, centrifuge tubes, microplate reader, distilled water (deionized water or ultrapure water is acceptable).

Index Determination

It is recommended to select 1-3 representative samples with significant differences for pre-experiment to familiarize with operating procedures, and adjust sample concentration properly to avoid unnecessary waste of samples and reagents.

1. Sample Extraction

① Tissue samples: Weigh about 0.1 g tissue sample, add 1 mL normal saline or PBS buffer for homogenization. Transfer all crude extract into EP tube, centrifuge at 12000 rpm at room temperature for 10 min, and collect supernatant for detection.

② Liquid samples: Clear liquid samples can be detected directly; turbid samples need centrifugation before taking supernatant for assay.

③ Bacterial & cell samples: Collect bacteria or cells into centrifuge tubes and discard supernatant after centrifugation. Take approximately 5 million bacteria/cells and resuspend in 1 mL normal saline or PBS. Perform ultrasonic disruption in ice bath (power: 200 W, ultrasonic for 3 s, interval 10 s, repeat 30 times). Centrifuge at 12000 rpm, 4 °C for 10 min, collect supernatant and place on ice for later use.

Note: For increased sample dosage, extract samples at the ratio of cell number (10^4 cells) : absolute ethanol (mL) = 500~1000 : 1.

Cell lysis buffer can also be used for sample treatment following the official protocol.

2. Detection Procedures

① Turn on the microplate reader, set temperature at 37 °C (skip temperature setting if unavailable), and adjust detection wavelength to 546 nm.

② Select 2 samples to confirm the optimal dilution factor (D) before formal detection.

③ Thaw all reagents to room temperature, and add reagents into 96-well plate in sequence.

Component (μL)	Test tube	Blank tube (single run)	Standard tube (single run)
Sample	6	-	-
Distilled water	-	6	-
Standard	-	-	6
Reagent 1	180	180	180
Mix and incubate at 37 °C for 5 min; read absorbance A1 at 546 nm.			
Reagent 2	60	60	60
Mix and incubate at 37 °C for 5 min; read absorbance A2 at 546 nm. ΔA = A2 - A1.			

Notes:

If the ΔA value of test well exceeds 0.5, dilute the sample with distilled water and bring dilution factor D into calculation formula.

If ΔA is lower than 0.005, increase sample loading volume V1 (e.g. from 6 μL to 10 μL or more, reduce Reagent 2 volume correspondingly; blank and standard wells shall be adjusted in the same way) or increase sample weight W. Recalculate results with revised parameters.

Result Calculation

1. Calculated by sample weight

Creatinine content (nmol/g) = (C_{std} × V_2) × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) ÷ (V_1 ÷ V × W) × D = 442 × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) ÷ W × D

2. Calculated by protein concentration

Creatinine content (nmol/mg prot) = (C_{std} × V_2) × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) ÷ (V_1 ÷ V × C_{pr}) × D = 442 × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) ÷ C_{pr} × D

3. Calculated by volume

Creatinine content (μmol/L) = (C_{std} × V_2) × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) ÷ V_1 × D = 442 × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) × D

4. Calculated by cell number

Creatinine content (μmol/10^4 cells) = (C_{std} × V_2) × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) ÷ (500 × V_1 ÷ V) × D = 0.884 × (ΔA_{test} - ΔA_{blank}) ÷ (ΔA_{std} - ΔA_{blank}) × D

Parameter Explanation:

C_{std}: Creatinine standard concentration, 0.05 mg/mL = 442 μmol/L = 442 nmol/mL.

Mr: Molecular weight of creatinine, 113.

V_1: Sample loading volume, 0.006 mL.

V_2: Standard solution loading volume, 0.006 mL.

V: Total extraction solution volume, 1 mL.

500: Ten thousand cell unit.

W: Sample weight (g).

D: Dilution factor; set as 1 without dilution.

C_{pr}: Sample protein concentration (mg/mL); BCA protein assay kit is recommended for determination.

Recommended Applications

Determination of creatinine content in tissue samples, liquid samples, bacterial samples, and cell samples using a 96-well microplate colorimetric procedure at 546 nm.

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