Creatinine (Cr) Colorimetric Assay Kit (Sarcosine Oxidase Method)

Catalog No: E-BC-K188-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 9.4 μmol/L

Detection range: 38.2-800 μmol/L

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

This kit can be used to detect the creatinine (Cr) content in serum, plasma, urine samples.

▲ Background

Creatinine (2-amino-1-methyl-2-imidazolidin-4-ketone) is a metabolite formed from creatine and phosphocreatine. Creatine and phosphocreatine are converted to creatinine in a non-enzymatic manner, and creatinine enters the bloodstream and is filtered by the glomerulus and excreted by the kidneys. Creatinine is found mainly in muscles, heart, brain and photoreceptor cells of retina.

▲ Detection principle

Creatinine (Cr) can be catalyzed by creatinase and generates creatine. Creatine can be hydrolyzed into sarcosine and urea by creatinase. The sarcosine can be catalyzed by sarcosine oxidase and form glycine, formaldehyde and hydrogen peroxide. The reaction between hydrogen peroxide, 2,4-(6-Tri-iodine-3-hydroxybenzoic acid) and 4-ampyrone can be catalyzed by peroxidase and form pink compound. Creatinine content can be calculated indirectly by measuring the OD value at 515 nm.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Solution A	20 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 2	Enzyme Solution B	7 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 3	1 mmol/L Standard Solution	1.5 mL × 2 vials	2-8℃ , 3 months
	Microplate	96 wells	
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

Materials prepared by users

Instruments

Microplate reader (510-520 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The Cr content of mouse serum (plasma) is low, it is recommended take 12 μL of sample to the reaction. Meanwhile, the recommended dilution gradient of standard is 0.4, 0.3, 0.25, 0.2, 0.15, 0.1, 0.05, 0 mmol/L and the volume of standard added to reaction is 12 μL.
2. The incubation time should be accurately.
3. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Pre-assay preparation

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (38.2-800 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human urine	20-30
Human serum	1
Rat serum	1
Porcine serum	1

Note:The diluent is normal saline (0.9% NaCl).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	515nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]：A - H, standard wells; S1 - S80, sample wells.

▲Operating steps

The preparation of standard curve

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 mmol/L.

The measurement of samples

1) Standard wells: Add 6 μL of standard solution with different concentrations to the corresponding wells.

   Sample wells: Add 6 μL of sample to the corresponding wells.

2) Add 180 μL of reagent 1 to each well and incubate at 37℃ for 5 min.
3) Add 60 μL of reagent 2 to each well, incubate at 37℃ for 2 min and measure the OD value (A1) of each well at 515 nm.
4) Incubate at 37℃ for 3 min and measure the OD value (A2) of each well at 515 nm. Calculate ΔA = A2-A1.

▲Operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	6	
Sample (μL)		6
Reagent 1 (μL)	180	180
Incubate at 37℃ for 5 min.		
Reagent 2 (μL)	60	60
Incubate at 37℃ for 2 min and measure the OD value (A1) of each well at 515 nm.		
Incubate at 37℃ for 3 min and measure the OD value (A2) of each well at 515 nm. Calculate ΔA = A2-A1.		

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

$$\text{Cr content } (\mu mol/L) = (\Delta A_{515} - b) \div a \times 1000^{*} \times f$$

Note:

y: $\Delta A_{Standard} - \Delta A_{Blank}$.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

ΔA_{515}: $OD_{Sample} - OD_{Blank}$.

1000*: Unit conversion, 1 mmol/L= 1000 μmol/L

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	38.2-800 μmol/L	Average intra-assay CV (%)	1.4
Sensitivity	9.4 μmol/L	Average inter-assay CV (%)	3.7
Average recovery rate (%)	106		

▲Example analysis

Dilute the human urine sample with normal saline for 20 times, then take 6 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.1734 x – 0.002, the average ΔA of the sample is 0.132, the average ΔA of the blank is 0.007, and the calculation result is:

$$\text{Cr content } (\mu mol/L) = \frac{0.132 - 0.007 + 0.002}{0.1734} \times 1000 \times 20 = 14648\ \mu mol/L$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.