（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K188-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（510-520 nm）

Detection range：20.45-400 μmol/L

Elabscience® Creatinine (Cr) Colorimetric Assay Kit

(Sarcosine Oxidase Method)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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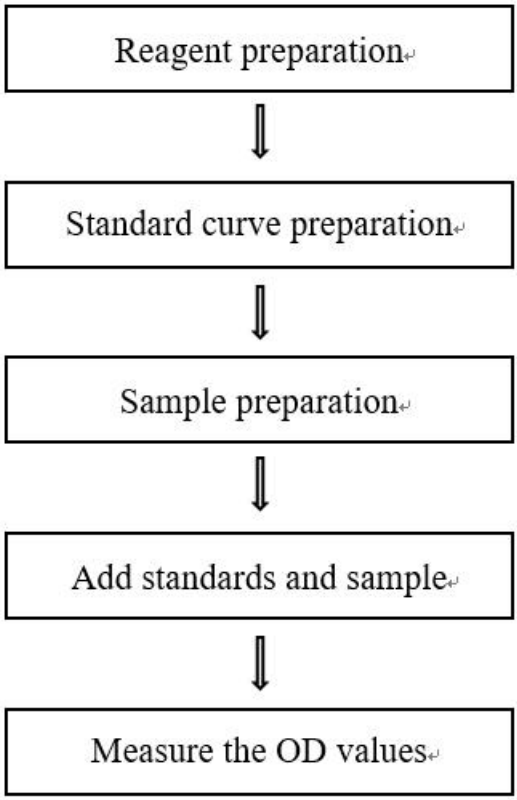
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Solution A	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Enzyme Solution B	3.5 mL × 1 vial	7 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	1 mmol/L Standard Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months
	Microplate	96 wells		No requirement
	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (510-520 nm, optimum wavelength: 515 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0.35**	**0.4**
1 mmol/L standard (μL)	0	20	30	40	50	60	70	80
Double distilled water (μL)	200	180	170	160	150	140	130	120

Sample preparation

① Sample preparation:

Serum (plasma) samples: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Urine: Collect fresh urine and centrifuge at 10000×g for 10 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

② Dilution of sample

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

Sample type	Dilution factor
Human urine	40-60
Human serum	1
Rat serum	1
Porcine serum	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

② If the number of samples is large，the multichannel pipeter is recommended to shorten the time and reduce the error between wells.

③ It is recommended to use the multichannel pipeter when the number of samples is large. It's better to measure no more than 20 sample wells at same time when there is no multichannel pipeter.

Operating steps

① Standard wells: Add 12 μL of standard solution with different concentrations to the corresponding wells.
Sample wells: Add 12 μL of sample to the corresponding wells.

② Add 180 μL of enzyme solution A to each well and incubate at 37°C for 5 min.

③ Add 60 μL of enzyme solution B to each well, incubate at 37°C for 2 min and measure the OD value (A_1) of each well at 515 nm.

④ Incubate at 37°C for 8 min and measure the OD value (A_2) of each well at 515 nm. Calculate $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Serum (plasma) and other liquid samples:

Definition: The amount of CS in 1 g tissue or cell protein per 1 minute that produce 1 μmol CoA at 37 °C is defined as 1 unit.

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

[Note]

f: Dilution factor of sample before test.

ΔA_{515}: $OD_{Sample} - OD_{Blank}$.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	88.60	271.00	350.00
%CV	1.7	1.5	1.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	88.60	271.00	350.00
%CV	3.2	3.7	4.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 106%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.13	0.22	0.34
Observed Conc. (mmol/L)	0.1	0.2	0.4
Recovery rate (%)	108	106	104

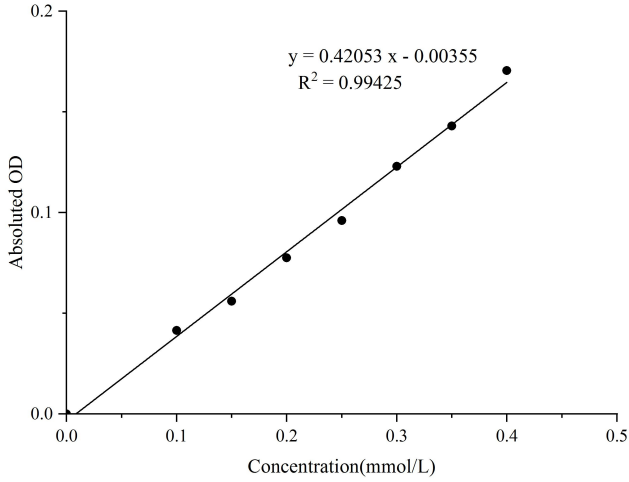
Sensitivity

The analytical sensitivity of the assay is 3.8 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (mmol/L)	0	0.1	0.15	0.2	0.25	0.3	0.35	0.4
Average ΔA	0.001	0.042	0.057	0.078	0.097	0.124	0.144	0.171
Absoluted OD	0.000	0.042	0.056	0.078	0.096	0.123	0.143	0.171



Appendix II Example Analysis

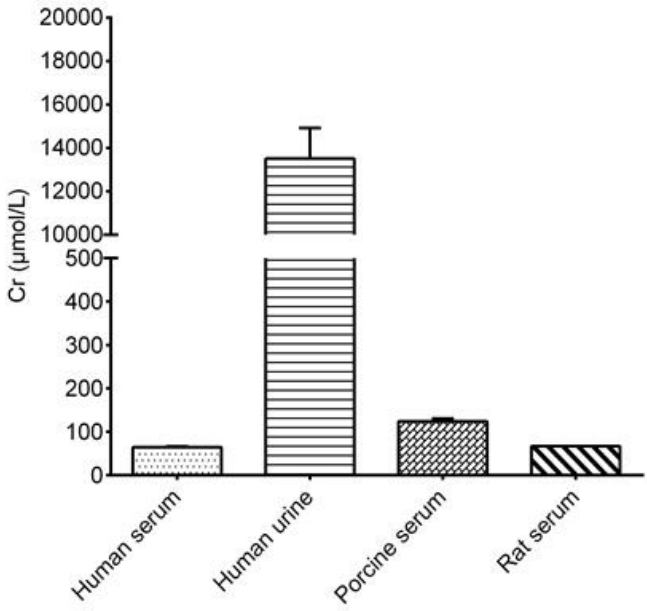
Example analysis:

Take 12 μL of mouse serum, carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.4205 x – 0.004, the average ΔA of the sample is 0.008, the average ΔA of the blank is 0.001, and the calculation result is:

$$\text{Cr content (μmol/L)} = (0.008 - 0.001 + 0.004) \div 0.4205 \times 1000 = 26.16 \text{ μmol/L}$$

Detect human serum, human urine (dilute for 40 times), porcine serum and rat serum according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.