Technical Manual

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

- Catalogue Code: MAES0138
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

23.03-800 μmol/L

Sensitivity:

3.9 μmol/L

Storage:

2-8°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use:

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

4. 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.

5. Kit Components & Storage:

Item	Specification	Storage
Enzyme Solution A	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Enzyme Solution B	7 mL × 1 vial	Avoid direct sunlight
Standard Solution (1 mmol/L)	1.5 mL × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (510-520 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

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

2. If there is a large sample number then a multichannel pipettor is recommended to shorten the time and reduce the error between wells.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation:

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

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

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (23.03-800 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
Mouse serum	1
Rat serum	1
Porcine serum	1

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

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 515 nm

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.

10. Operation 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 well:** Add 10 μL of standard solution with different concentrations to the corresponding wells.
   Sample well: Add 10 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 $\Delta A = A_2 - A_1$.

Operation Table

	Standard well	Sample well
Standard solution with different concentrations (μL)	10	
Sample (μL)		10
Enzyme solution A (μL)	180	180
Incubate at 37°C for 5 min.		
Enzyme solution B (μL)	60	60
Incubate at 37°C for 2 min and measure the OD value (A_1) of each well at 515 nm.		
Incubate at 37°C for 8 min and measure the OD value (A_2) of each well at 515 nm. Calculate $\Delta A = A_2 - A_1$.		

11. Calculations:

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.

Serum (plasma) and other liquid sample:

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

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}: $\Delta A_{Sample} - \Delta A_{Blank}$.
1000*: Unit conversion, 1 mmol/L= 1000 μmol/L

Email: info@assaygenie.com **Web:** www.assaygenie.com

12. Performance Characteristics:

Detection Range	23.03-800 μmol/L
Sensitivity	3.9 μmol/L
Average recovery rate (%)	106
Average inter-assay CV (%)	3.7
Average intra-assay CV (%)	1.4

Analysis

Take 10 μL of human serum, carry out the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.4472 x – 0.005, the average ΔA of the sample is 0.034, the average ΔA of the blank is 0.004, and the calculation result is:

$$\text{Cr content (μmol/L)} = (0.034 - 0.004 + 0.005) \div 0.4472 \times 1000$$
$$= 78.26 \text{ μmol/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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