TECHNICAL MANUAL

Creatinine (Cr) Colorimetric Assay Kit (Sarcosine Oxidase)

- **SKU CODE:** MAES0138
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure OD value at two time points
- Calculate delta A
- Calculate creatinine concentration

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Enzyme Solution A	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8 °C, 12 months, shading light
Reagent 2	Enzyme Solution B	3.5 mL x 1 vial	7 mL x 1 vial	35 mL x 1 vial	2-8 °C, 12 months, shading light

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 3	1 mmol/L Standard Solution	1.5 mL x 1 vial	1.5 mL x 2 vials	15 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of the 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 to create 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 dilution table: Item ①②③④⑤⑥⑦⑧; 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.

7. Sample preparation

1. **Sample preparation:** Serum (plasma) samples: detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for one month. Urine: Collect fresh urine and centrifuge at 10,000 x g for 10 min at 4 °C. Take the supernatant and preserve on ice for detection. If not detected on the same day, urine can be stored at -80 °C for one month.

2. **Dilution of samples:** The recommended dilution factors for different samples are as follows (for reference only): Human urine: 40-60, Human serum: 1, Rat serum: 1, Porcine serum: 1. Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Prevent the formation of bubbles when transferring the supernatant into the microplate.
2. If the number of samples is large, a multichannel pipetter is recommended to shorten the time and reduce error between wells.
3. It is recommended to use a multichannel pipetter when the number of samples is large. It's better to measure no more than 20 sample wells at the same time when there is no multichannel pipetter.

9. 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 (A1) of each well at 515 nm.
4. Incubate at 37 °C for 8 min and measure the OD value (A2) of each well at 515 nm. Calculate ΔA = A2 - A1.

10. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding 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:

Cr content (μmol/L) = (ΔA_515 - b) ÷ a × 1000* × f

[Note]

f: Dilution factor of sample before test.

ΔA_515: OD_{Sample} – OD_{Blank}.

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

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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

Recovery

Three samples of high concentration, middle concentration, and low concentration were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 106%.

Recovery

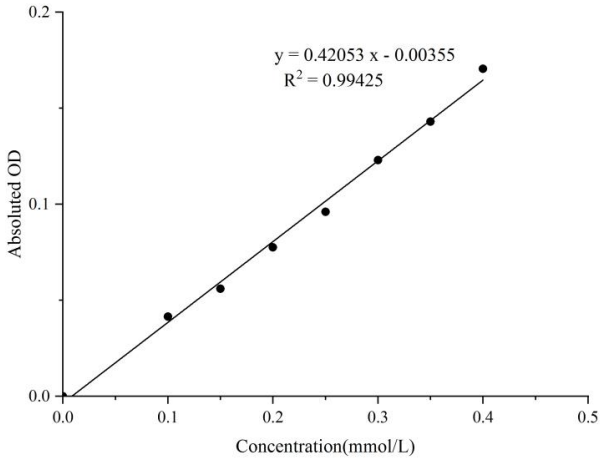
Standard 1	Standard 2	Standard 3
0.13	0.22	0.34
0.1	0.2	0.4
108	106	104

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.

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), the standard curve and data are provided below for reference only.



Standard curve

Concentration (mmol/L)	A1 value	A1 value	Average A1	A2	A2	Average A2	Average ΔA	Absolute OD
0	0.066	0.066	0.061	0.067	0.067	0.062	0.001	0.000
0.1	0.070	0.070	0.072	0.114	0.114	0.114	0.042	0.042

Concentration (mmol/L)	A1 value	A1 value	Average A1	A2	A2	Average A2	Average ΔA	Absolute OD
0.15	0.076	0.076	0.078	0.132	0.132	0.135	0.057	0.056
0.2	0.084	0.084	0.086	0.161	0.161	0.164	0.078	0.078
0.25	0.091	0.091	0.090	0.188	0.188	0.187	0.097	0.096
0.3	0.096	0.096	0.097	0.215	0.215	0.221	0.124	0.123
0.35	0.104	0.104	0.108	0.247	0.247	0.252	0.144	0.143
0.4	0.118	0.118	0.116	0.291	0.291	0.287	0.171	0.171

11. Appendix II Example Analysis

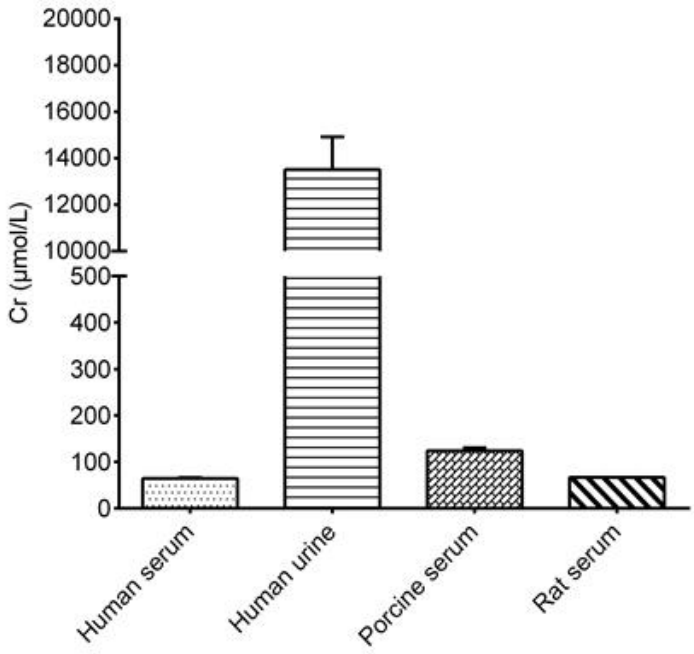
Example analysis:

Take 12 μL of mouse serum and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.4205x - 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:

Cr content (μmol/L) = (0.008 - 0.001 + 0.004) ÷ 0.4205 × 1000 = 26.16 μmol/L

Detect human serum, human urine (diluted 40 times), porcine serum, and rat serum according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.