



TECHNICAL MANUAL

Sodium (Na) Colorimetric Assay Kit

- **SKU CODE:** MAES0212
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate and measure absorbance
- Calculate sodium content

2. Intended use

This kit can be used to measure sodium ion content in serum, plasma, and tissue samples.

3. Detection principle

β -galactosidase activated by sodium ions catalyzes glycoside analogues to produce glycoside and o-nitrophenol. The increase in absorbance is measured at 405 nm, and the sodium ion content is calculated indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent	5 mL x 1 vial	10 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 2	Enzyme Stock Solution	10 mL x 1 vial	20 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Enzyme Reagent	Powder x 1 vial	Powder x 2 vials	2-8 °C, 12 months
Reagent 4	10 mmol/L Standard	1.6 mL x 1 vial	1.6 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Incubator, Microplate reader (405 nm)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** Dissolve one vial of enzyme reagent with 8 mL of enzyme stock solution. Store at 2-8°C for 1 day.

7. Sample preparation

Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
2. Wash tissue in cold PBS (0.01 M, pH 7.4).
3. Homogenize 20 mg tissue in 180 µL double distilled water with a Dounce homogenizer at 4°C.
4. Centrifuge at 10,000 x g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample

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

Human serum: 9-12

Human plasma: 9-12

Mouse serum: 9-12

Rat plasma: 10-15

Note: The diluent is double distilled water. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

The sample needs to be diluted before detection because of the high sodium content.

9. Operating steps

1. Blank well: Add 10 μ L of double distilled water to the corresponding well. Standard well: Add 10 μ L of standard to the corresponding well. Sample well: Add 10 μ L of sample to the corresponding well.
2. Add 80 μ L of chromogenic agent to each well.
3. Add 120 μ L of working solution to each well.
4. Measure the OD value of each well at 405 nm with microplate reader, recorded as A1.
5. Incubate at 37°C for 3 min, measure the OD value of each well at 405 nm with microplate reader, recorded as A2, $\Delta A = A_2 - A_1$.

10. Calculation

Serum (plasma) sample:

$$\text{Na}^+ \text{ content (mmol/L)} = (\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}}) / (\Delta A_{\text{Standard}} - \Delta A_{\text{Blank}}) \times c \times f$$

2. Tissue samples:

$$\text{Na}^+ \text{ content (mmol/gprot)} = (\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}}) / (\Delta A_{\text{Standard}} - \Delta A_{\text{Blank}}) \times c \div C_{\text{pr}} \times f$$

[Note]

ΔA_{Sample} : The OD value of sample well, $A_2 - A_1$.

ΔA_{Blank} : The OD value of blank well, $A_2 - A_1$.

$\Delta A_{\text{Standard}}$: The OD value of standard well, $A_2 - A_1$.

c: The concentration of the standard: 10 mmol/L

C_{pr} : Concentration of protein in sample, gprot/L

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

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 (mmol/L)	2.50	9.70	15.60
%CV	2.5	2.1	2.0

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 (mmol/L)	2.50	9.70	15.60
%CV	8.2	8.5	8.5

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	4.6	12.5	17.3
Observed Conc. (mmol/L)	4.6	11.8	17.5
Recovery rate (%)	99	94	101

Sensitivity

The analytical sensitivity of the assay is 0.02 mmol/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.

12. Appendix II Example Analysis

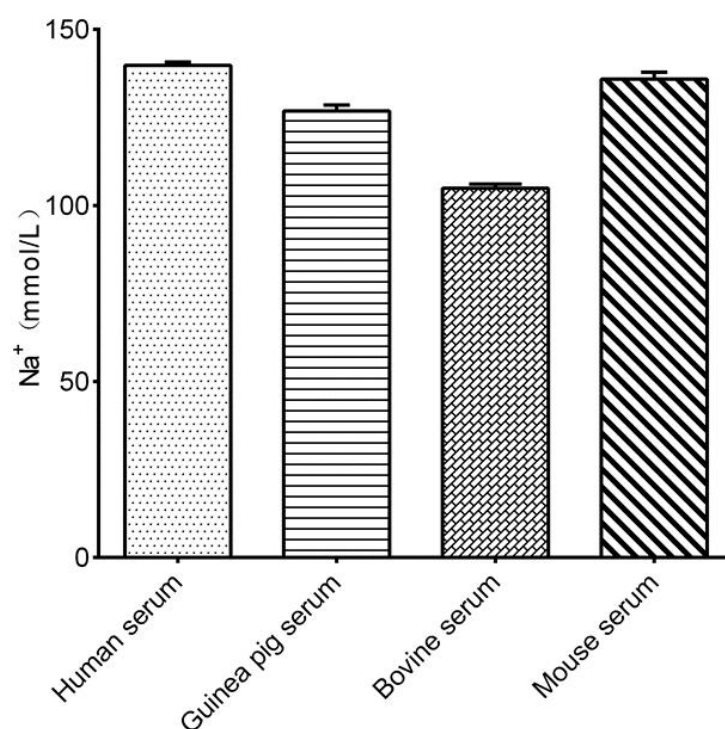
Example analysis:

For human serum, take 10 μL of human serum diluted 10 times, and carry out the assay according to the operation steps. The results are as follows:

The average change OD value of sample (ΔA_{Sample}) is 0.433, the average change OD value of blank (ΔA_{blank}) is 0.146, the average change OD value of standard ($\Delta A_{\text{standard}}$) is 0.351, and the calculation result is:

$$\text{Na}^+ \text{ content (mmol/L)} = (0.433 - 0.146) \div (0.351 - 0.146) \times 10 \times 10 = 140 \text{ mmol/L}$$

Detect human serum (diluted 10 times), guinea pig serum (diluted 10 times), bovine serum (diluted 10 times) and mouse serum (diluted 10 times), according to the protocol, the result is as follows:



13. 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!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



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