Technical Manual

Sodium (Na) Colorimetric Assay Kit

- Catalogue Code: MAES0145
- Size: 100 Assays (96 samples)
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method (405 nm)

Specification:

100 Assays (96 samples)

Range:

0.02-10 mmol/L

Storage:

2-8°C for 12 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 12 months.

Do not use components from different batches of kit.

2. Intended Use

This kit can be used for detecting the concentration of sodium ions in serum samples.

3. Detection Principle

The formation of nitrophenol from the substrate nitropyranoside is catalyzed by sodium-activated β-galactosidase. The increase rate of absorbance value of nitrophenol at 405 nm per unit time was proportional to the sodium concentration.

4. Kit components & storage

Item	Specification	Storage
Reagent 1: Chromogenic Agent	50 mL / 1 vial	Store at 2–8 °C for up to 12 months. Protect from direct light.
Reagent 2: Enzyme Stock Solution	40 mL × 2 vials	
Reagent 3: Enzyme Reagent	Powder × 4 vials	
Reagent 4: 5mmol/L Standard	1.6 mL x 1 vial	

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.

5. Instruments:

Spectrophotometer (405 nm), Biochemical analyzer (405 nm), Microplate reader (405 nm), Micropipettor, Vortex mixer, Centrifuge, Water bath, Incubator.

6. Reagent preparation:

Bring all reagents to room temperature before use.

Preparation of working solution:

Dilute on vial of enzyme reagent with 15 mL of enzyme stock solution. Sote at 2-8°C for 2 days.

7. Sample Preparation

Plasma or serum samples:

Test directly after dilution, samples can be stored at -80ºC for a month.

Tissue samples:

1- Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
2- Homogenize 20 mg tissue in double distilled water with a dounce homogenizer at 4℃.
3- Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.
4- Meanwhile, determine the protein concentration of supernatant

- **Sample dilutions (recommended)**

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

Sample type	Dilution factors
Human Serum	15-25
Mouse Serum	15-25
Human Plasma	15-25
Rat Plasma	15-25
10% Mouse Liver tissue homogenate	15-25
10% Rat Heart tissue homogenate	15-25

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

* High sodium content of the samples required dilution for determination

8. Operating steps

1- **Blank tube**: Take 50 μL of double distilled water to the 2 mL EP tube.
Standard tube: Take 50 μL of 5 mmol/L standard to the 2 mL EP tube.
Sample tube: Take 50 μL of sample to the 2 mL EP tube.

2- Add 400 μL of chromogenic agent to each tube, mix fully.

3- Add 600 μL of working solution to each tube, mix fully.

4- Set the spectrophotometer to zero with double distilled water and measure the absorbance at 405 nm with 1 cm optical path quartz cuvette, as A_1.

5- Incubate at 37°C for 3 min, measure the absorbance of each tube at the wavelength 405 nm with 1 cm optical path quartz cuvette, as A_2, $\Delta A = A_2 - A_1$. Note: When removing liquid with the pipetting gun, be careful to avoid bubbles. The incubation time should be counted from the measurement of well A1. Due to the short incubation time, it is recommended to process one tube at a time.

9. Calculations

Sample:

1- Serum and plasma samples:

$$Na^+content\ \left(\frac{mmol}{L}\right) = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times c \times f$$

2- Tissue sample:

$$Na^{+}content\ \left(\frac{mmol}{gprot}\right) = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times c \div C_{pr} \times f$$

$\Delta A_{sample:}$ The change of OD value of sample tube, A2-A1

ΔA blank: The change OD value of blank tube, A2-A1

ΔA standard: The change OD value of standard tube, A2-A1

c: The concentration of standard solution, 5 mmol/L

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

f: Dilution factor of sample before test.

Appendix 1 Performance Characteristics

1- Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.50	3.50	6.50
%CV	2.3	4.5	2.8

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

2- Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.50	3.50	6.50
%CV	7.5	8.9	7.0

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

3- Recovery

	Sandard 1	Sandard 2	Sandard 3
Expected Conc. (mmol/L)	2.50	3.50	6.50
Observed Conc. (mmol/L)	2.30	3.40	6.20
Rcovery rate(%)	92	98	95

Select three samples with high, medium, and low concentrations, and test each concentration in six parallel replicates to obtain an average recovery rate of 95%.

4- 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.

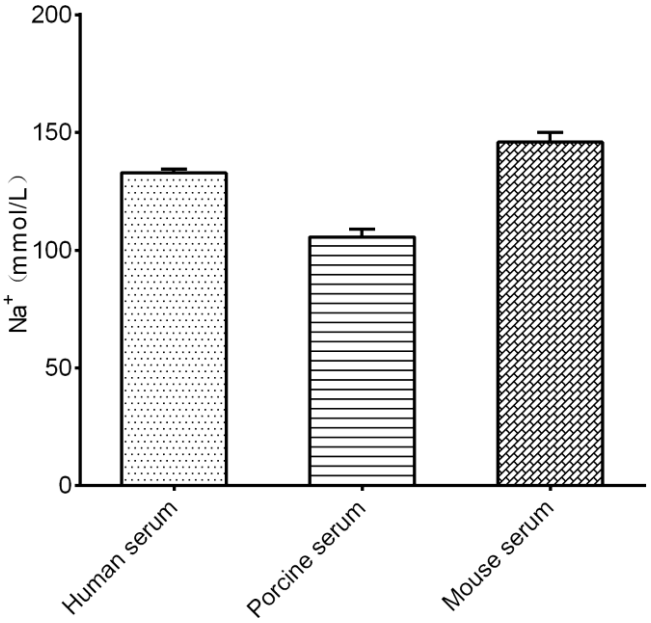
Appendix 2: Example Analysis

Example analysis：

Take 50 μL of human serum which dilute for 20 times and carry the assay according to the operation steps. The results are as follows:
The ΔA_{sample} of the sample tube is 0.434, the ΔA_{blank} of the blank tube is 0.146, the $\Delta A_{standard}$ of the standard tube is 0.356, and the calculation result is:

$$Na^{+}content\left(\frac{mmol}{L}\right) = \frac{0.434 - 0.146}{0.356 - 0.146} \times 5 \times 20 = 137.14\, mmol/L$$

Detect human serum (dilute for 20 times), porcine serum (dilute for 20 times), mouse serum (dilute for 20 times) 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. Assay Genie 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.

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.

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