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

Catalog No：E-BC-K207-S

Specification：50 Assays(46 samples)/100 Assays(96 samples)

Measuring instrument：Spectrophotometer (405 nm)

Detection range：0.02 - 10 mmol/L

Elabscience® Sodium (Na^+) Colorimetric Assay Kit

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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 5

The key points of the assay 6

Operating steps 7

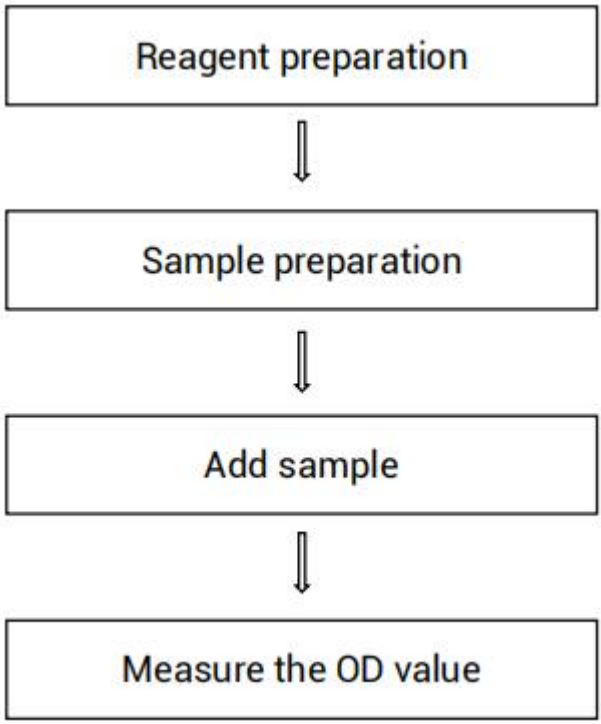
Calculation 8

Appendix I Performance Characteristics 9

Appendix П Example Analysis 10

Statement 11

Assay summary



Intended use

This kit can be used to measure sodium (Na^+) content in serum, plasma and animal tissue samples.

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.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Chromogenic Agent	25 mL × 1 vial	50 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Enzyme Stock Solution	40 mL × 1 vial	40 mL × 2 vials	2-8℃, 12 months
Reagent 3	Enzyme Reagent	Powder × 2 vials	Powder × 4 vials	2-8℃, 12 months
Reagent 4	5 mmol/L Standard	1.6 mL × 1 vial	1.6 mL × 1 vial	2-8℃, 12 months

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:

Spectrophotometer (405 nm)

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of working solution：

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

Sample preparation

① 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 (E-BC-K318-M).

② **Dilution of sample**

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

Sample type	Dilution factor
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.

The key points of the assay

The high sodium content of the samples required dilution for determination.

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 the liquid is removed by the pipetting gun, be careful to suction to avoid bubbles. The incubation time should start from the measurement of A_1. Because of the short incubation time, one tube at a time was recommended.

Calculation

The sample:

1. Serum and plasma samples:

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

2. Tissue sample:

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

[Note]

ΔA_{sample}: The change of OD value of sample tube, A_2-A_1

ΔA_{blank}: The change OD value of blank tube, A_2-A_1

$\Delta A_{standard}$: The change OD value of standard tube, A_2-A_1

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 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 (mmol/L)	2.50	3.50	6.50
%CV	2.3	4.5	2.8

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 (mmol/L)	2.50	3.50	6.50
%CV	7.5	8.9	7.0

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 95%.

	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

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 Π Example Analysis

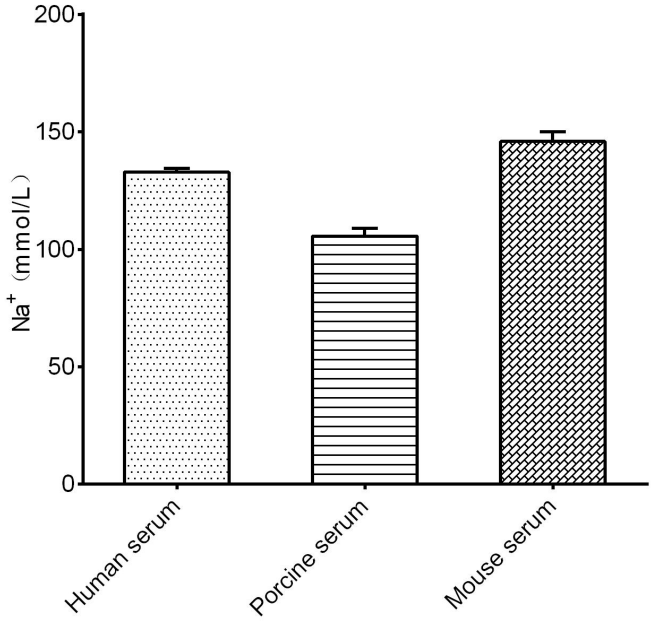
Example analysis：

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:

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

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. 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.