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

Catalog No：E-BC-K207-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader (405 nm)

Detection range：0.02-20 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.

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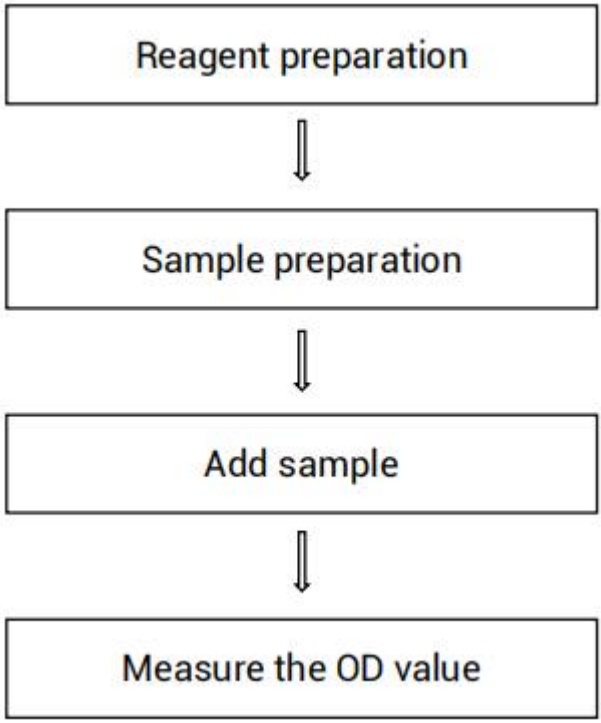
Assay summary



Intended use

This kit can be used to measure sodium ions content in serum (plasma) and tissue samples.

Detection principle

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

Kit components & storage

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

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:

Incubator, Microplate reader (405 nm)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The 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.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a 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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ 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	9-12
Human plasma	9-12
Mouse serum	9-12
Rat plasma	10-15

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

Operating steps

① Blank well: add 10 μL of double distilled water to the corresponding well.
Standard well: add 10 μL of standard to the corresponding well.
Sample well: add 10 μL of sample to the corresponding well.

② Add 80 μL of chromogenic agent to each well.

③ Add 120 μL of working solution to each well.

④ Measure the OD value of each well at 405 nm with microplate reader, recorded as A_1.

⑤ Incubate at 37℃ for 3 min, measure the OD value of each well at 405 nm with microplate reader, recorded as A_2, $\Delta A=A_2-A_1$.

Calculation

The sample:

1. Serum (plasma) sample:

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

2. Tissue samples:

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

[Note]

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

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

$\Delta A_{Standard}$: 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.

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	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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.50	9.70	15.60
%CV	8.2	8.5	8.5

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 98%.

	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.

Appendix Π Example Analysis

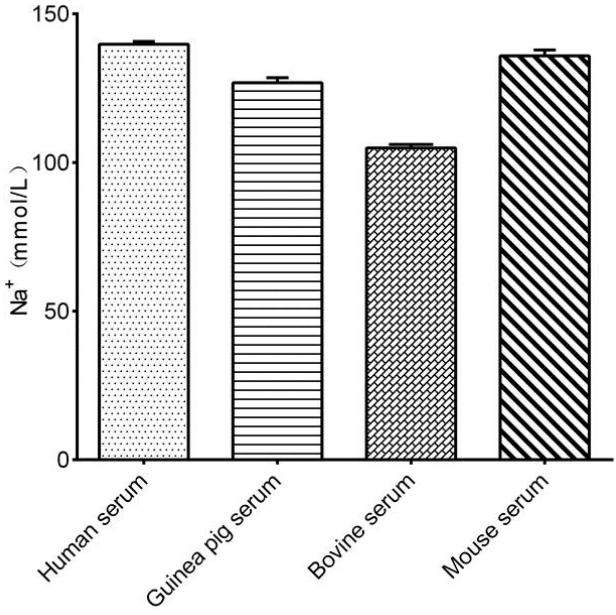
Example analysis：

For human serum, take 10 μL of human serum diluted for 10 times, and carry 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 ($\Delta A_{standard}$) is 0.351, and the calculation result is:

Na^+ content (mmol/L) = (0.433 - 0.146) ÷ (0.351 - 0.146) × 10 × 10 = 140 mmol/L

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



Appendix III Publications

1. Sun T, Hui J, Lin B, et al. Sequential biofluid sampling microfludic multi-sensing patch for more accurate sweat analysis under sedentary condition[J]. Applied Materials Today, 2023, 34: 101910.
2. Lin B, Li F, Hui J, et al. Modular Reconfigurable Approach Toward Noninvasive Wearable Body Net for Monitoring Sweat and Physiological Signals[J]. ACS sensors, 2024.
3. Siboto A, Akinnuga A M, Khumalo B, et al. Ameliorative Effects of a Rhenium (V) Compound with Uracil-Derived Ligand Markers Associated with Hyperglycaemia-Induced Renal Dysfunction in Diet-Induced Prediabetic Rats[J]. International Journal of Molecular Sciences, 2022, 23(23): 15400.
4. Yu S, He Z, Gao K, et al. Dioscorea composita WRKY12 is involved in the regulation of salt tolerance by directly activating the promoter of AtRCI2A[J]. Plant Physiology and Biochemistry, 2023, 196: 746-758.
5. Lv H, Niu J, Pan W, et al. Stool-softening effect and action mechanism of free anthraquinones extracted from Rheum palmatum L. on water deficit-induced constipation in rats[J]. Journal of Ethnopharmacology, 2024, 319: 117336.
6. Wang X, Huang Y, Sun X, et al. Protective Role of Anthraquinone Purpurin Against Cardiotoxicity Induced by Isoproterenol in Adult Wistar Rats[J]. Pharmacognosy Magazine, 2025, 21(1): 256-268.

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.