Technical Manual

Sodium (Na) Colorimetric Assay Kit

- **Catalogue Code: MAES0145**
- **Size: R1: 20mL (×2)**
  R2: 10mL (×2)
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

R1: 20mL (×2)
R2: 10mL (×2)

Range:

80-180 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.

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2. Intended Use

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

3. Detection Principle

Production of o-nitrophenol and galactose by o-nitrophenol-β-D-galactoside (ONPG) catalyzed by sodium dependent β-D-galactosidase. The amount of o-nitrophenol is directly proportional to the concentration of sodium ion in the sample. The o-nitrophenol is yellow in alkaline environment. The increase of absorbance is determined at 405 nm, and the content of sodium ion is calculated indirectly.

4. Kit components & storage

Item	Specification	Storage
Enzyme Working Solution (Components: Good's Buffer, Phenol, 4-AAP, Cholesterol esterase, Cholesterol oxidase, Peroxidase)	30 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Cholesterol Standard (5.17 mM)	0.2 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (495-525 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)
- Isopropanol

5. Assay Notes:

1. Protect the reagent from contamination of glucose, cholesterol, etc.
2. Prevent the formation of bubbles when the reagents are added into the microplate.
3. When measuring low content samples such as cells, the volume of sample should be increased to 5~10 μL, and the volume of blank well and standard well should be increased at the same time.

6. Reagent preparation:

Bring all reagents to room temperature before use.

7. Sample Preparation

Sample requirements: Reducing substances such as ascorbic acid and glutathione should not be added to the sample.

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Cell culture supernatant:
Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80°C for a month.

4. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

5. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

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Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (80-180 mmol/L).

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

Sample Type:	Dilution Factor:
Human plasma	1
Mouse plasma	1
Rat plasma	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
HepG2 cells	1

Note: The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH7.4); The diluent of animal tissue and cells is isopropanol.

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank wells; B, standard wells; S1-S92, sample wells.

9. Operation Steps

The preparation of standard curve

Dilute H_2O_2 standard (1 mol/L) with double distilled water to a serial concentration.

The measurement of samples

1. Add 100 μL of buffer solution to each well and preheat at 37°C for 10 min.
2. **Standard well:** add 15 μL of standards with different concentrations to the corresponding wells.
   Sample well: add 15 μL of sample to the corresponding wells.
3. Add 100 μL of ammonium molybdate reagent and mix fully.
4. Mix for 5 s with microplate reader and stand for 10 min at room temperature.
5. Measure the OD values of each well at 405 nm with microplate reader.

Operation Table

	Blank well	Standard well	Sample well
Double distilled water (μL)	2.5		
5.17 mM Cholesterol Standard (μL)		2.5	
Sample (μL)			2.5
Enzyme Working Solution (μL)	250	250	250
Mix thoroughly, incubate at 37°C for 10 min, measure the OD value at 510 nm with microplate reader.			

10. Calculations

1. Serum (plasma) and other liquid sample:

$$\text{TC content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

$$\text{TC content (mmol/g fresh weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

3. Cell sample:

$$\text{TC content } (\mu\text{mol}/10^6 \text{ cells}) = \frac{\Delta A_1}{\Delta A_2} \times c \div \frac{N}{V} \times f$$

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

c: The concentration of standard, 5.17 mmol/L

f: Dilution factor of sample before tested

m: The weight of tissue sample, mg

V: The volume of isopropanol, mL

N: The number of cells. For example, the number of cells is $5*10^6$, N is 5

11. Performance Characteristics

Detection Range	80-180 mmol/L
Sensitivity	
Average recovery rate (%)	
Average inter-assay CV (%)	
Average intra-assay CV (%)	

Analysis

Take 2.5 μL of human serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.239, the average OD value of the blank is 0.049, the average OD value of the standard is 0.295, and the calculation result is:

$$\text{Total Cholesterol content (mmol/L)} = \frac{0.239-0.049}{0.295-0.049} \times 5.17 \times 1 = 3.99 \text{ mmol/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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