Technical Manual

Potassium (K) turbidimetric Assay Kit

- **Catalogue Code: MAES0170**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Turbidimetric method

Specification:

96T

Range:

0.01-0.80 mmol/L

Sensitivity:

0.002 mmol/L

Storage:

2-8°C for 6 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 6 months.

Do not use components from different batches of kit.

2. Background

Potassium ions are vital for the functioning of all living cells. The transfer of potassium ions across nerve cell membranes is necessary for normal nerve transmission; potassium deficiency and excess can each result in numerous signs and symptoms, including an abnormal heart rhythm and various electrocardiographic abnormalities. Fresh fruits and vegetables are good dietary sources of potassium. The body responds to the influx of dietary potassium, which raises serum potassium levels, with a shift of potassium from outside to inside cells and an increase in potassium excretion by the kidneys.

3. Intended Use

This kit can be used to measure Potassium (K) content in serum, plasma, milk, tissue, cells and other samples.

4. Detection Principle

Under the alkaline condition, the sodium tetraphenylborate reacts with the potassium ions in the sample to form the potassium tetraphenylborate which is white and small particles with small solubility. Potassium tetraphenylborate particles are in a stable suspension state in the solution. The turbidity is proportional to the potassium ion concentration in the sample and potassium content can be calculated indirectly by measuring the OD value at 450 nm.

5. Kit Components & Storage

Item	Specification	Storage
Precipitant A	20 mL × 1 vial	2-8°C, 6 months
Precipitant B	1.25 mL × 2 vials	2-8°C, 6 months
Chromogenic Agent A	12.5 mL × 2 vials	2-8°C, 6 months
Chromogenic Agent B	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Potassium Standard (1 mmol/L)	1.25 mL × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Centrifuge
- Microplate Reader (450-600 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Deionized water

6. Assay Notes:

1. Haemolysed samples should not be adopted since red blood cells contain high concentrations of potassium ions.

2. Ammonia, mercury and chlorine can interfere with the determination of the potassium ion.

3. It is recommended to use deionized water to prepare tissue homogenates and prevent potassium ion pollution.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **protein precipitant**: Mix the precipitant A and precipitant B with the ratio of 8:1. Prepared the fresh solution before use.

3. Preparation of **chromogenic agent**: Dissolve a vial of chromogenic agent B with 12.5 mL chromogenic agent A and mix fully. Prepared the fresh solution before use.

8. Sample Preparation

Sample requirements:

1. Since there is a high concentration of potassium ions in red blood cells, this sample type would prevent hemolysis.

2. Deionized water is the best medium for tissue homogenization. It prevents the contamination of potassium ions.

3. Ammonium ions, heavy metal ions and chloride ions will affect the reaction, so these sample can’t be added.

4. The samples are stable at 2~8°C for 3~4 days and stable below -20°C for several months.

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) and preserve on ice before 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) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Milk sample:
Collect the milk sample, centrifuge at 10000 g for 10 min at 4°C and collect middle layer liquid for measurement.

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 (4×10^6): deionized water (μL) =1: 400. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before 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. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of deionized water (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 and preserve on ice before 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.

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 (0.01-0.80 mmol/L).

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

Sample Type:	Dilution Factor:
Human serum	1
Rat serum	1
RAW 264.7 cellular supernatant	1
Human plasma	1
Human milk	1
10% Rat liver tissue homogenization	2-4

Note: The diluent is deionized water.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

Plate Set Up:

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

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 1 mmol/L Potassium Standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 mmol/L.

The measurement of samples

1. Preparation of supernatant: Mix the sample and protein precipitant with the ratio of 1:9 (For example, take 20 µL of sample and 180 µL of protein precipitant to mix fully). Centrifugate at 1100 g for 10 min. Take supernatant for detection

2. **Standard well:** Take 50 µL of standard solution with different concentrations to the wells.
   Sample well: Take 50 µL of supernatant to the wells.

3. Add 200 µL of chromogenic agent into the wells of Step 2.

4. Cover the plate sealer, mix fully and stand for 5 min at room temperature.

5. Measure the OD value at 450 nm with microplate reader.

Operation Table

	Standard well	Sample well
Standard solution of different concentrations (µL)	50	
Sample supernatant (µL)		50
Chromogenic agent (µL)	200	200
Mix fully and stand for 5 min. Measure the OD value at 450 nm.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: y= ax + b..

1. Serum (plasma) and other liquid sample:

$$\text{Potassium content (mmol/L)} = \frac{\Delta A_{450} - b}{a} \times 10 \times f$$

2. Tissue sample:

$$\text{Potassium content (mmol/gprot)} = \frac{\Delta A_{450} - b}{a} \times 10 \times f \div C_{pr}$$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA_{450}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
10: Dilution multiple of sample in preparation of supernatant.
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	0.01-0.80 mmol/L
Sensitivity	0.002 mmol/L
Average recovery rate (%)	94
Average inter-assay CV (%)	6.1
Average intra-assay CV (%)	1.1

Analysis

Take 0.1 g of fresh rat liver sample, add 0.9 mL of 2-8°C deionized water, then homogenize treat the sample in ice water bath, centrifuge at 10000 g for 10 min at 4°C, then dilute the supernatant with deionized water for 2 times and carry the assay according to the operation table.

The results are as follows:
standard curve: y = 0.77073 x – 0.00139, the average OD value of the sample is 0.404, the average OD value of the blank is 0.045, the concentration of protein in sample is 9.23 gprot/L, and the calculation result is:

$$\textbf{K}^{+}\textbf{ content (mmol/gprot)} = (0.404 - 0.045 + 0.00139) \div 0.77073 \times 10 \times 2 \div 9.23$$

$$= 1.01 \text{ mmol/gprot}$$

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.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

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