



TECHNICAL MANUAL

Potassium (K) turbidimetric Assay Kit

- **SKU CODE:** MAES0170
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and sample
- Measure the OD value

2. Intended use

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

3. Detection principle

Under alkaline conditions, sodium tetraphenylborate reacts with potassium ions in the sample to form potassium tetraphenylborate, which appears as white particles with low solubility. The potassium tetraphenylborate particles remain 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.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Precipitant A	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8 °C, 12 months
Reagent 2	Precipitant B	1.25 mL x 1 vial	1.25 mL x 2 vials	12.5 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Chromogenic Agent A	12.5 mL x 1 vial	12.5 mL x 2 vials	12.5 mL x 10 vials	2-8 °C, 12 months
Reagent 4	Chromogenic Agent B	Powder x 1 vial	Powder x 2 vials	Powder x 10 vials	2-8 °C, 12 months, shading light

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 5	1 mmol/L Potassium Standard	1.25 mL x 1 vial	1.25 mL x 2 vials	12.5 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments: Microplate reader (450-600 nm), Centrifuge, Micropipettor, Vortex mixer

Reagents: Double distilled water

6. Reagent preparation

- 1. Preparation of protein precipitant:** For each sample, prepare 180 µL of protein precipitant by mixing 160 µL of precipitant A with 20 µL of precipitant B. Mix well. The protein precipitant should be prepared fresh immediately before use.
- 2. Preparation of chromogenic agent:** Dilute one vial of chromogenic agent B with 12.5 mL of chromogenic agent A and mix well. The chromogenic agent should be prepared fresh immediately before use.
- 3. Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L potassium standard with double distilled water to create a serial concentration series. The recommended dilution gradient is: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 mmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not analyzed on the same day, serum or plasma can be stored at -80 °C for one month.

Urine: Collect fresh urine and centrifuge at 10,000 x g for 15 min at 4 °C. Take the supernatant and preserve on ice for detection.

Milk sample: Collect the milk sample and centrifuge at 10,000 x g for 10 min at 4 °C. Collect the middle layer liquid for measurement.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
2. Wash tissue in cold deionized water.
3. Homogenize 20 mg tissue in 180 μ L deionized water with a dounce homogenizer at 4 °C.
4. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
2. Wash cells with deionized water.
3. Homogenize 1×10^6 cells in 200 μ L deionized water with an ultrasonic cell disruptor at 4 °C.
4. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

- Human serum: 1
- Rat serum: 1
- RAW 264.7 cellular supernatant: 1
- Human plasma: 1
- Human milk: 1
- 10% Rat liver tissue homogenate: 2-4

Note: The diluent is deionized water. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Hemolyzed samples should not be used since red blood cells contain high concentrations of potassium ions.
2. Ammonia, mercury, and chlorine can interfere with the determination of potassium ions.

3. It is recommended to use double distilled water to prepare tissue homogenate and avoid potassium ion contamination.

9. Operating steps

1. Preparation of supernatant: Mix the sample and protein precipitant at a ratio of 1:9 (for example, take 20 μ L of sample and 180 μ L of protein precipitant and mix thoroughly). Centrifuge at 1,100 x g for 10 min. Take the supernatant for detection.
2. Standard well: Add 50 μ L of standard solution with different concentrations to the wells. Sample well: Add 50 μ L of supernatant to the wells.
3. Add 200 μ L of chromogenic agent to each well.
4. Cover with the plate sealer, mix thoroughly and incubate for 5 min at room temperature.
5. Measure the OD value of each well at 450 nm with a microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.
3. Plot the standard curve using the absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve ($y = ax + b$) with graph software (or Excel).

The sample:

1. Serum (plasma) and other liquid samples:

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

2. Tissue sample:

$$\text{Potassium content (mmol/g}_{\text{prot}}) = (\text{delta}A_{450} - b) \div a \times 10 \times f \div C_{\text{pr}}$$

[Note]

10: Dilution multiple of sample in preparation of supernatant.

f: Dilution factor of sample before test.

C_{pr} : Concentration of protein in sample ($\text{g}_{\text{prot}}/\text{L}$)

deltaA: Absolute OD ($\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}$).

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.46	0.65
%CV	1.3	1.2	0.8

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.46	0.65
%CV	5.8	6.4	6.1

Recovery

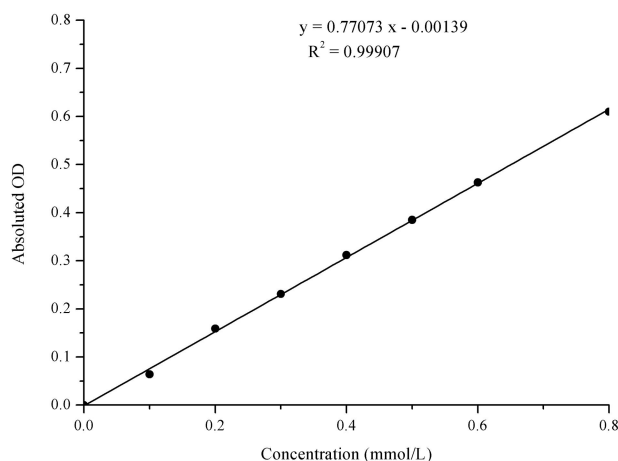
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.35	0.55
Observed Conc. (mmol/L)	0.1	0.3	0.5
Recovery rate (%)	94	92	96

Sensitivity

The analytical sensitivity of the assay is 0.002 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.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	Average OD	Absolute OD
0	0.044	0
0.1	0.108	0.064
0.2	0.203	0.159
0.3	0.275	0.231
0.4	0.356	0.312
0.5	0.429	0.385
0.6	0.507	0.463
0.8	0.654	0.610

12. Appendix II Example Analysis

Example analysis:

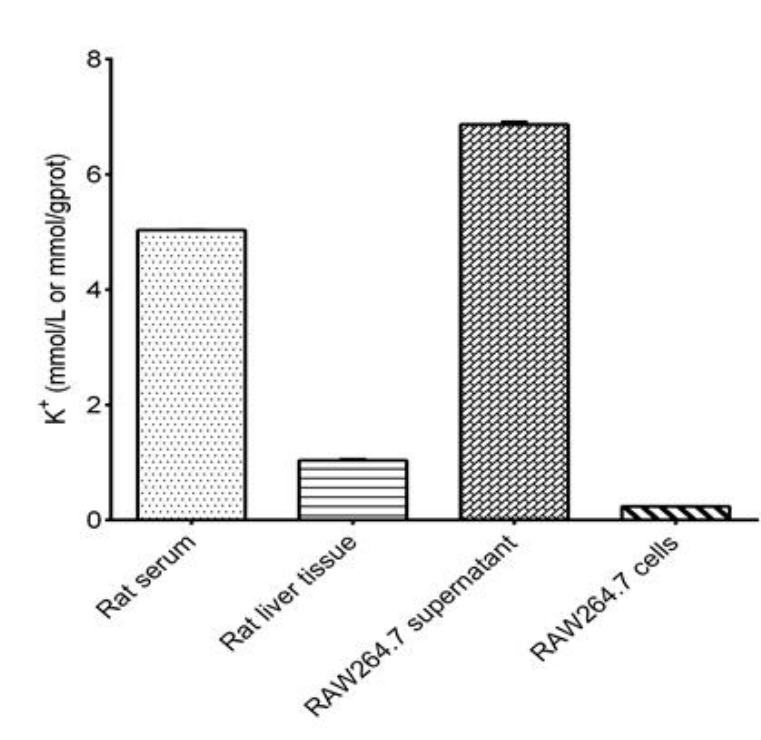
Take 0.1 g of fresh rat liver sample, add 0.9 mL of 2-8 °C double distilled water, then homogenize the sample in an ice water bath. Centrifuge at 10,000 x g for 10 min at 4 °C,

then dilute the supernatant with deionized water 2-fold and perform the assay according to the operation steps. The results are as follows:

Standard curve: $y = 0.77073x - 0.00139$, 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 the sample is 9.23 g_{prot}/L, and the calculation result is:

$$K^+ \text{ content (mmol/g}_{\text{prot}}) = (0.404 - 0.045 + 0.00139) \div 0.77073 \times 10 \times 2 \div 9.23 = 1.01 \text{ mmol/g}_{\text{prot}}$$

Detect rat serum (V = 20 μL), 10% rat liver tissue homogenate (protein concentration is 9.23 g_{prot}/L, diluted 2-fold, V = 20 μL), culture supernatant of RAW264.7 cells, and RAW264.7 cells (protein concentration is 4.40 g_{prot}/L, V = 20 μL) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.