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

Catalog No：E-BC-K279-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（450-600 nm）

Detection range：0.01-0.80 mmol/L

Elabscience® Potassium (K) Turbidimetric 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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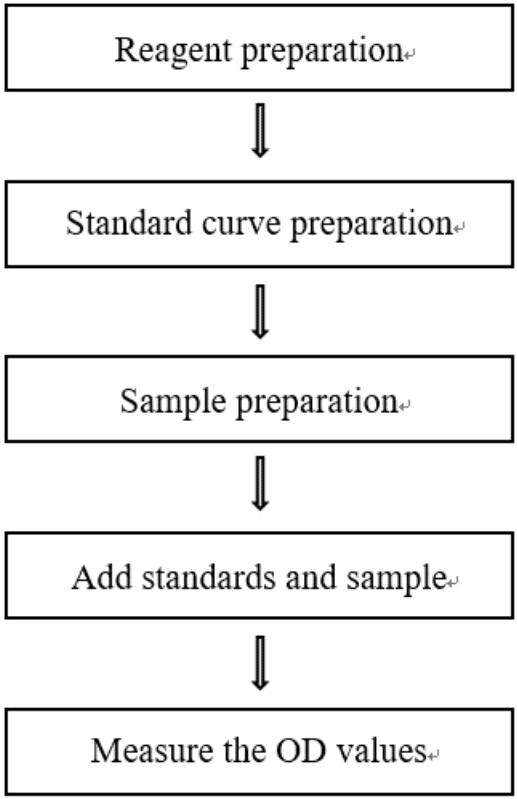
Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Precipitant A	10 mL ×1 vial	20 mL ×1 vial	2-8 ℃, 12 months
Reagent 2	Precipitant B	1.25 mL ×1 vial	1.25 mL ×2 vials	2-8 ℃, 12 months
Reagent 3	Chromogenic Agent A	12.5 mL ×1 vial	12.5 mL ×2 vials	2-8 ℃, 12 months
Reagent 4	Chromogenic Agent B	Powder ×1 vial	Powder ×2 vials	2-8 ℃, 12 months shading light
Reagent 5	1 mmol/L Potassium Standard	1.25 mL ×1 vial	1.25 mL ×2 vials	2-8 ℃, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

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

Reagents:

Double distilled water

Reagent preparation

① The preparation of protein precipitant:

For each sample, prepare 180 μL of protein precipitant (mix well 160 μL of precipitant A, and 20 μL of precipitant B). The protein precipitant should be prepared on spot.

② The preparation of chromogenic agent:

Dilute one vial of chromogenic agent B with 12.5 mL of chromogenic agent A, mix well. The chromogenic agent should be prepared on spot.

③ The 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 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.8**
1 mmol/L potassium standard (μL)	0	25	50	75	100	125	150	200
Double distilled water (μL)	250	225	200	175	150	125	100	50

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.

Urine: collect fresh urine and centrifuge at 10000×g for 15 min at 4℃. Take the supernatant and preserve it on ice for detection.

Milk sample: collect the milk sample and centrifuge at 10000×g for 10 min at 4℃ and collect 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 μL deionized water with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes 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).

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 μL deionized water with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes 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	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. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

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

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

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

Operating steps

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 to each well

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

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

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 absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent 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 sample:

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

2. Tissue sample:

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

[Note]

10: Dilution multiple of sample in preparation of supernatant.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L)

ΔA: Absolute OD ($OD_{Sample} - OD_{Blank}$).

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)	0.25	0.46	0.65
%CV	1.3	1.2	0.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

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

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 94%.

	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

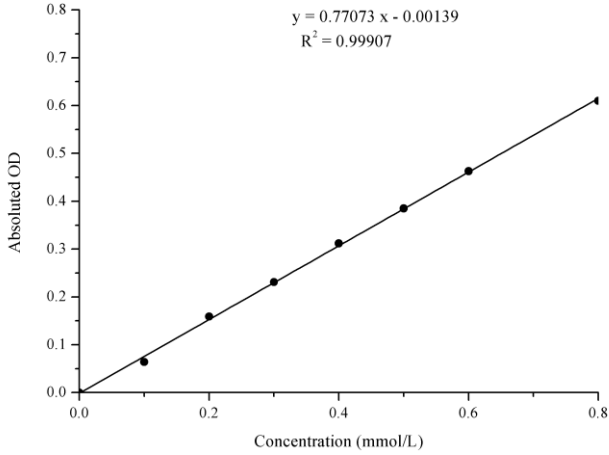
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

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



Appendix II Example Analysis

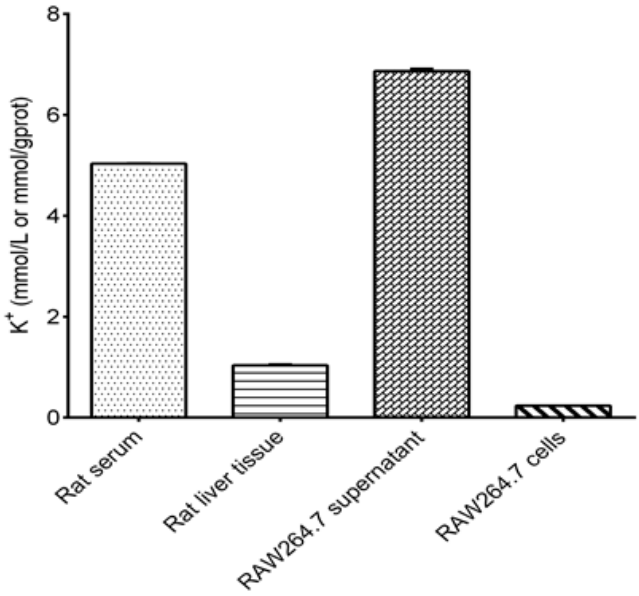
Example analysis:

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

K^+ content (mmol/gprot) = (0.404-0.045+0.00139) ÷0.77073 ×10 ×2 ÷9.23= 1.01 mmol/gprot

Detect rat serum (V=20 μL), 10% rat liver tissue homogenate (the concentration of protein is 9.23 gprot/L, dilute for 2 times, V=20 μL), culture supernatant of RAW264.7 cells and RAW264.7 cells (the concentration of protein is 4.40 gprot/L, V=20 μL) 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.