Potassium (K) turbidimetric Assay Kit

Catalog Number EEA018 (96 tests)

Rev 1.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

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.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)

Precipitant A	20 mL
Precipitant B	1.25 mL ×2 vials
Chromogenic Agent A	12.5 mL ×2 vials
Chromogenic Agent B	Powder ×2 vials
1 mmol/L Potassium Standard	1.25 mL ×2 vials
96 wells Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- Since there is a high concentration of potassium ions in red blood cells, the sample should avoid hemolysis.
- Deionized water is the best homogenized medium for tissue homogenization to prevent the contamination of potassium ions.
- Ammonium ion, heavy metal ion, chloride ion will affect the reaction, so the sample can't be added.
- The samples are stable at 2~8℃ for 3~4 days and stable below -20℃ for several months.

Serum, plasma and milk samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with homogenization medium at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of deionized water: the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month

Cells:

- Collect the cells and wash the cells with homogenization medium for 1 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 (10^6): deionized water =1: 300-500.
- Sonicate or grind with hand-operated in ice water bath.

- Centrifuge at 10000 g for 10 min at 4℃, then take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).
If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.01-0.80 mmol/L).
Note: The diluent is deionized water.
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

Preparation of protein precipitant

Prepare fresh solution before use by mixing 8 parts of precipitant A with 1 part of precipitant B.

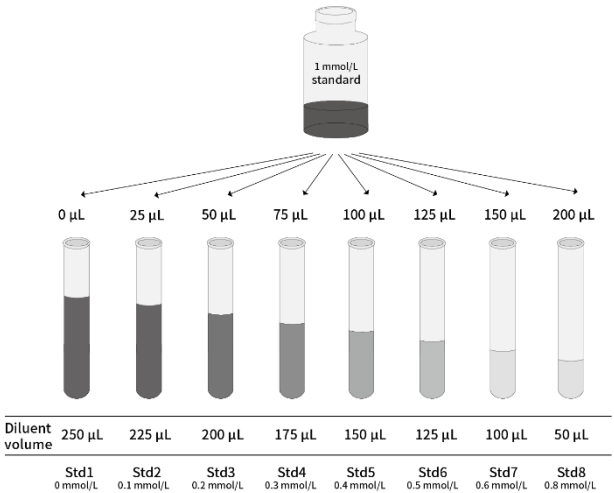
Preparation of chromogenic agent

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

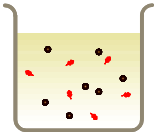
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 1 mmol/mL ptassium standard with distilled or deionized 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.



Assay Procedure

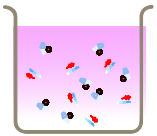


Preparation of supernatant: Mix the sample and protein precipitant with the ratio of 1:9 (For example, take 20 μL of sample and 180 μL of protein precipitant to mix fully). Centrifugate at 1100 g for 10 min. Take supernatant for detection.

1. **Add sample**

a. Standard well: add 50 μL of Standard solution with different concentrations.

b. Sample well: add 50 μL of supernatant.



2. **Add substrate**

a. Add 200 μL of chromogenic agent into the wells

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

c. Measure the OD values of each well with microplate reader at 450 nm

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) 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).

Serum (plasma) and other liquid sample:

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

Tissue and cells sample:

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

[Note]

y: The absolute OD value of standard;

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve;

ΔA_{450}: $OD_{sample} - OD_{Control}$;

f: Dilution factor of sample before test;

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

10: Dilution multiple of sample in preparation of supernatant.

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

Take 50 μL of 10% rat liver tissue homogenization(dilute for 2 times) and carry the assay according to the operation table. The results are as follows:

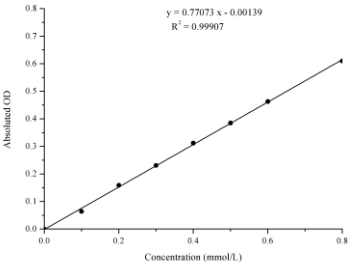
standard curve: y = 0.77073 x – 0.00139, the average OD value of the sample is 0.404, the average OD value of the blank is 0.045, the concentration of protein in sample is 9.23 gprot/L, and the calculation result is:

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

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–0.80 mmol/L standard.

Concentration (mmol/L)	OD at 450 nm	Standard Curve
0.80	0.654	
0.60	0.507	
0.50	0.429	
0.40	0.356	
0.30	0.275	
0.20	0.203	
0.10	0.108	
0.00	0.044	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.20	0.40	0.60
%CV	6.6	6.2	5.5

CV = Coefficient of Variation

- Intra-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)	0.20	0.40	0.60
%CV	0.8	1.3	1.2

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples

Sample Type	Range (**mmol/L**)	Average (**mmol/L**)
Serum	3.5-5	4.25
Plasma (EDTA and heparin)	3.1-4.7	3.9

▪ 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 97.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （mmol/L）	0.2	0.4	0.6
Observed Conc. （mmol/L）	0.192	0.388	0.594
Recovery rate (%)	96	97	99

▪ Recommended 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												

- 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.

Limited product warranty

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