Potassium (K) Assay Kit

Catalog No.: abx298870

Size: 96 tests

Detection range: 0.01 mmol/L – 0.80 mmol/L

Sensitivity: 0.002 mmol/L

Storage: Store the kit components in the dark at 2-8°C.

Application: For Potassium concentration measurement in serum, plasma, milk, tissue homogenates, cell lysates, and other biological fluids.

Introduction

Potassium ions are vital for all living cells. Potassium ion transfer across cell membranes is essential for normal nerve transmission, potassium deficiency or excess can result in an abnormal heart rhythm and various electrocardiographic abnormalities. The body will respond to an influx of dietary potassium, such as fruit and vegetables, which subsequently raises serum potassium levels, increasing potassium excretion by the kidneys. Under an alkaline condition, sodium tetraphenylborate reacts with potassium ions to form a potassium tetraphenylborate. Potassium tetraphenylborate particles are in a stable suspension within the solution.

Abbexa's Potassium Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Potassium concentration. The product has an absorbance maxima at 450 nm. The intensity of the color is proportional to the Potassium activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Precipitation Solution A: 20 ml
3. Precipitation Solution B: 2 × 1.25 ml
4. Detection Reagent A: 2 × 12.5 ml
5. Detection Reagen B: 2 vials
6. Potassium Standard (1 mmol/L): 2 × 1.25 ml
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Deionized water
3. Pipette and pipette tips
4. Vials/tubes
5. Sonicating water bath
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Precipitation Solution:** Mix Precipitation Solution A and Precipitation Solution B at a ratio of 8:1. Prepare the required volume fresh before use.
- **Detection Reagent:** Dissolve a vial of Detection Reagent B with 12.5 ml of Detection Reagent A and mix fully. Ensure the Detection Reagent B has dissolved completely. Prepare immediately before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 25°C for 30 mins. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Milk:** Collect the sample and centrifuge at 10000 x g for 4°C for 10 mins. Collect the middle liquid layer for measurement.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with deionized water at 2-8°C. Absorb the water with filter paper and weigh. For each 1 g of tissue, add 9 ml of homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 1500 × g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with homogenization medium 1-2 times. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of homogenization medium per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately, or aliquot and store at -80°C for up to 1 month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

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

Sample Type	Dilution Factor
Human Serum	1
Rat Serum	1
RAW264.7 Cell Supernatant	1
Human Plasma	1
Human Milk	1
10% Rat Liver Tissue Homogenate	2-4

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- The diluent is deionized water.
- Samples should be free of hemolysis, ammonium ions, chloride ions, ammonium ions and potassium ions, as these will interfere with the assay.

B. Assay Procedure

1. Set standard and sample wells on the microplate and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Dilute 1 mmol/L Potassium Standard with deionized water to a serial concentration. The recommended gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 mmol/L. Please see the below table as a reference.

Standard concentrations (mmol/L)	1 mmol/L standard solution (µl)	Deionized water (µl)
0.0	0	250
0.1	25	225
0.2	50	200
0.3	75	175
0.4	100	150
0.5	125	125
0.6	150	100
0.8	200	50

3. To prepare the supernatant, mix the sample and Precipitation Solution with a 1:9 ratio. Centrifuge at 1100 × g for 10 minutes. Take the supernatant for detection.
4. Take 50 µl of standard solution to the wells to the standard wells.
5. Take 50 µl of supernatant to the sample wells.

6. Add 200 µl of Detection Reagent Solution to all wells.
7. Cover with a plate sealer, mix fully and let stand at room temperature for 5 minutes.
8. Measure the OD value at 450 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{450} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). Create the standard curve with graph software. The Potassium concentration of the samples can be interpolated from the standard curve. If the sample has been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

1. Serum, plasma, and cell culture supernatant samples:

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

2. Tissues samples:

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

where:

y	$OD_{Standard} - OD_{Blank}$
x	The concentration of standard
a	The gradient of the standard curve
b	The intercept of the standard curve
10	Dilution multiple of sample in preparation of supernatant
f	Dilution factor of sample before test
$\mathrm{C_{pr}}$	Concentration of protein in sample (gprot/L)
ΔA_{450}	$(OD_{Sample} - OD_{Blank})$