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ab252904 Potassium Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab252904
(use https://www.abcam.cn/ab252904 for china, or https://www.abcam.co.jp/ab252904 for Japan)

For the measurement of potassium concentration in serum.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

The Potassium Assay Kit (ab252904) is a simple plate based assay kit for the measurement of potassium concentration in human serum samples. It is based on a potassium detection agent that is activated by potassium. The detection agent catalyzes a reaction between two substrates in presence of potassium, leading to the formation of an intermediate, which further reacts with the development enzyme mix/developer and oxidizes a non-fluorescent probe producing a strong, stable fluorescence signal.

The rate of the reaction is proportional to the potassium concentration present in the sample and can be monitored at Ex/Em= 535/587 nm. Sodium concentrations of up to 10 fold that of potassium, do not interfere with the assay. However, since serum has about 25-30 times higher sodium levels that potassium, it can slightly activate the potassium detection agent (although to a much lower extent than potassium).

A sodium masking agent has been included in our kit, which prevents any activation caused due to sodium present in serum delivering a highly specific assay for the detection of potassium. The kit can detect as low as 10 nmol of potassium per well and is linear up to 50 nmol.

2. Protocol Summary

Prepare all samples, standards and controls as instructed.

Add up to 2.5 µL of sample into a 96 well black plate; adjust final volume to 50 µL with Potassium Assay Buffer.

Create the 50 µL Reaction Mix and add to standards, controls and test samples.

Immediately start reading the fluorescence every 30 seconds at EX/EM 535/587 at room temperature in kinetic mode.

Calculate the rate of reaction and the potassium concentration in the test samples using the obtained RFU values.

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Potassium Assay Buffer	25 mL	-20°C	-20°C
Potassium Substrate I	1 vial	-20°C	-20°C
Potassium Substrate II	1 vial	-20°C	-20°C
Potassium Detection Mix/Potassium Detection Solution	1 vial	-20°C	-20°C
Development Enzyme Mix I/Potassium Developer	1 vial	-20°C	-20°C
OxiRed Probe/Potassium Probe	200 µL	-20°C	-20°C
Sodium Masking Reagent/Sodium Masking Solution	500 µL	-20°C	-20°C
Potassium Standard/Potassium Standard (5 mM)	500 µL	-20°C	-20°C

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-Well black plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader).

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Potassium Assay Buffer:

Warm to room temperature before use.

6.2 Potassium Substrate I and Substrate II:

Reconstitute with 220 µL water each. Aliquot and store at -20°C.

ΔNote: If precipitates are observed while thawing the reconstituted reagents, the thawed vials may be incubated in a water bath at 37°C for 20-30 minutes (until precipitates completely dissolve.

6.3 Potassium Detection Mix/Potassium Detection Solution and Deelopment Enzyme Mix I/Developer:

Reconstitute with 220 µL Potassium Assay Buffer each. Aliquot and store at -20°C.

6.4 OxiRed Probe/Potassium Probe and Sodium Masking Reagent/Sodium Masking Solution:

Thaw at room temperature before use. Protect form light. Aliquot and store the remaining
stock at -20°C in dark.

6.5 Potassium Standard/Potassium Standard (5mM):

Thaw at room temperature before use. Aliquot and store the remaining at -20°C.

7. Sample preparation

7.1 Serum:

- Serum samples can be used "as is" without any processing. We recommend using "off the clot" serum that is free from additives such as EDTA.
- Add up to 2.5 µL sample into each well of a 96 well black plate. Serum may be diluted with Potassium Assay buffer before addition to wells if higher than 10 mM potassium level is expected.
- Make up the volume to 50 µL with Potassium Assay Buffer.

8. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

8.1 Add 0, 2, 4, 6, 8, and 10 µL of the 5 mM working Potassium standard solution to a series of wells in duplicate in a 96-well plate.

8.2 Bring the total volume of each well to 50 µL with Potassium Assay Buffer to generate 0, 10, 20, 30, 40, and 50 nmol of Potassium per well.

Standard#	Potassium Standard/5 mM Potassium Standard (µL)	Potassium Assay Buffer (µL)	End amount of Potassium in well (nmol/well)
1	0	50	0
2	2	48	10
3	4	46	20
4	6	44	30
5	8	42	40
6	10	40	50

9. Assay Procedure

9.1 Reaction mix:

1. Prepare Reaction Mix immediately before running the assay.
2. Mix enough reagents for the number of assays to be performed.
3. Add Reaction Mix to all sample and standard wells. For each well, prepare 50 µL:

	Reaction Mix
Potassium Assay Buffer	35 µL
Potassium Substrate I	2 µL
Potassium Substrate II	2 µL
Development Enzyme Mix I/Potassium Developer	2 µL
Potassium Detection Mix/Potassium Detection Solution	2 µL
Sodium Masking Reagent/Sodium masking solution	5 µL
OxiRed Probe/Potassium Probe	2 µL

4. Mix and add 50 µL of the Reaction Mix to each well of the 96-well plate.
5. Have the plate reader ready at RT, at Ex/Em 535/587 nm on kinetic mode set to record fluorescence every 30 seconds.

9.2 Measurement:

1. Immediately start recording fluorescence at 30 second intervals for 30-40 minutes in kinetic mode.

ΔNote: Standards should also be read in kinetic mode.

9.3 Calculation:

1. Subtract the 0 standard reading from all other standard readings. Plot the Potassium standard curve.
2. Obtain the rate of reaction using the following equation:

$$\text{Rate of reaction} = (RFU_{t2} - RFU_{t1}) / (t_2 - t_1)$$

With t2 and t1 chosen in the reaction linear portion after initial lag (30 minutes).

3. Obtain the standard curve by plotting Potassium amount (nmol) on the x-axis and Rate of reaction for the respective potassium amount on the Y axis.
4. Apply background subtracted sample RFU to Standard Curve to get B nmol Potassium in the sample well.

Potassium concentration in sample: C = (B / V) X D (nmol/ml or µM)

Where:

B = Amount of Potassium in the sample well from the standard curve (nmol).

V = Sample volume added into the reaction well (in mL)

D = Sample dilution factor (in case serum was diluted before addition to reaction well).

10. Typical Data

Data provided **for demonstration purposes only**.

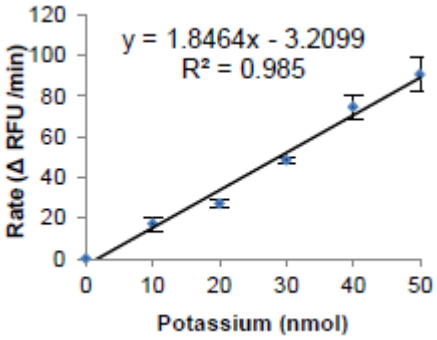

Figure 1. Potassium Standard curve

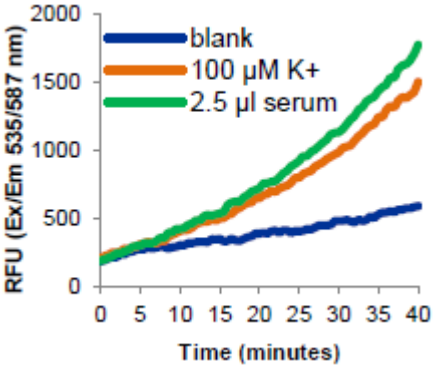

Figure 2. Reaction kinetics for 100 µM (10 nmol) potassium standard and for 2.5 µL "off-the-clot" human serum.

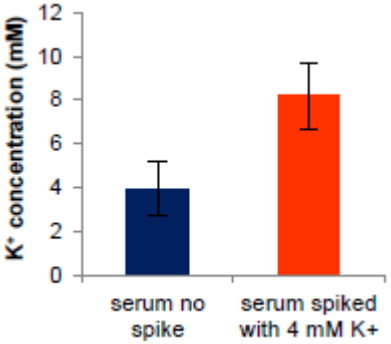

Figure 3. Estimated potassium concentrations in "off-the-clot" human serum. Spiked experiments show 99 ± 5 % recovery. Experiments were run according to kit protocol.

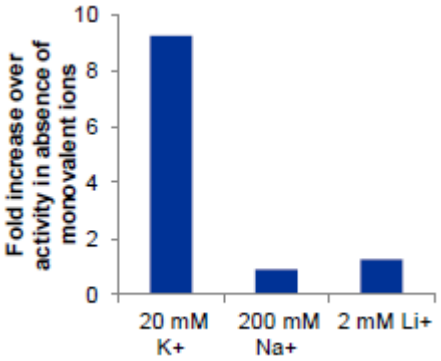

Figure 4. Sodium concentrations of up to 10 fold that of potassium do not interfere with the assay.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)