

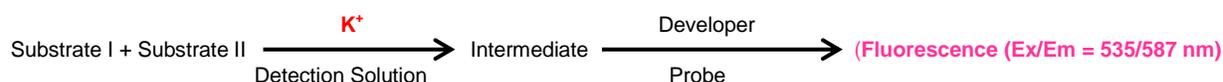
Potassium (Serum) Detection Assay Kit (Fluorometric)

rev 03/20

(Catalog # K940-100; 100 assays; Store at -20°C)

I. Introduction:

Potassium (K^+) is an essential micronutrient that regulates osmotic balance in the body along with sodium, calcium, magnesium, chloride and phosphate. It is required for various body functions such as muscle contraction, neural impulses and also as a co-factor for several enzymes. Blood potassium level is tightly regulated and ranges between 3.5 - 5.2 mM for healthy adults. Hypokalemia (low potassium levels) can lead to hypertension, whereas hyperkalemia (high serum potassium level) indicates heart problems or kidney failure. **BioVision's Potassium Assay Kit** 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 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 than 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 highly specific 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.



II. Applications:

Measurement of potassium concentration in serum

III. Sample Type:

- Serum

IV. Kit Contents:

Components	K940-100	Cap Code	Part Number
K Assay Buffer	25 ml	WM	K940-100-1
K Substrate I	1 vial	Orange	K940-100-2
K Substrate II	1 vial	Purple	K940-100-3
K Detection Solution	1 vial	Blue	K940-100-4
K Developer	1 vial	Green	K940-100-5
K Probe	200 μ l	Red	K940-100-6
Sodium Masking Solution	500 μ l	White	K940-100-7
K Standard (5 mM)	500 μ l	Yellow	K940-100-8

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

- **K Assay Buffer:** Warm to room temperature (RT) before use.
- **K Substrate I and Substrate II:** Reconstitute each vial with 220 μ l water each. Divide into aliquots and store at -20°C.
Note: If precipitates are observed while thawing the reconstituted reagents, the thawed vials may be incubated on a water bath at 37°C for 20-30 min (until the precipitates completely dissolves).
- **K Detection Solution and K Developer:** Reconstitute each vial with 220 μ l K Assay Buffer. Divide into aliquots and store at -20°C.
- **K Probe and Sodium Masking Solution:** Thaw at RT before use. Protect from light. Divide into aliquots and store the remaining stock at -20°C in dark.
- **K Standard (5 mM):** Thaw at RT before use. Divide into aliquots and store the remaining at -20°C.

VII. Potassium Detection Assay Protocol:

1. **Sample preparation:** 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 μ l sample into each well of a 96 well black plate. Serum may be diluted with K Assay buffer before addition to wells if higher than 10 mM potassium level is expected. Make up the volume to 50 μ l with K Assay Buffer.
2. **Standard Curve Generation:** Add 0, 2, 4, 6, 8 and 10 μ l of the provided K Standard (5 mM) into a series of wells in a black 96-well plate to obtain 10, 20, 30, 40 and 50 nmol/ well. Adjust the volume of each well to 50 μ l with K Assay Buffer.
3. **Reaction Mix:** Prepare reaction mix immediately before running the assay. Mix enough reagents for the number of assays to be performed. Add reaction Mix to all sample and Standard wells. For each well, prepare 50 μ l:
- 4.

<u>Reaction Mix</u>	
K Assay Buffer	35 μ l
K Substrate I	2 μ l
K Substrate II	2 μ l
K Developer	2 μ l
K Detection Solution	2 μ l
Sodium masking solution	5 μ l
K Probe	2 μ l

Add the reaction mix to all wells of the 96-well black plate.

Note:

Have the plate reader ready at RT, Ex/Em = 535/587 nm on kinetic mode set to record fluorescence every 30 sec.

4. Measurement: Immediately start recording fluorescence at 30 sec intervals for 30-40 min. **Standards should also be read in kinetic mode.**

5. Calculation: Subtract the blank (0 nmol Potassium) from K Standard (10-50 nmol) RFU values and sample RFU values. Obtain rate of reaction using the following equation:

$$\text{Rate of reaction} = (\text{RFU}_{t_2} - \text{RFU}_{t_1}) / (t_2 - t_1); \text{ with } t_2 \text{ and } t_1 \text{ chosen in the reaction linear portion after initial lag (30 min)}$$

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. Apply background subtracted sample RFU to Standard Curve to get B nmol Potassium in the sample well.

$$\text{Potassium concentration in sample: } C = (B / V) \times D \text{ (nmol/ml or } \mu\text{M)}$$

Where **B** = Amount of Potassium in the sample well from Standard Curve (nmol)

V = Volume of sample added into the well (ml)

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

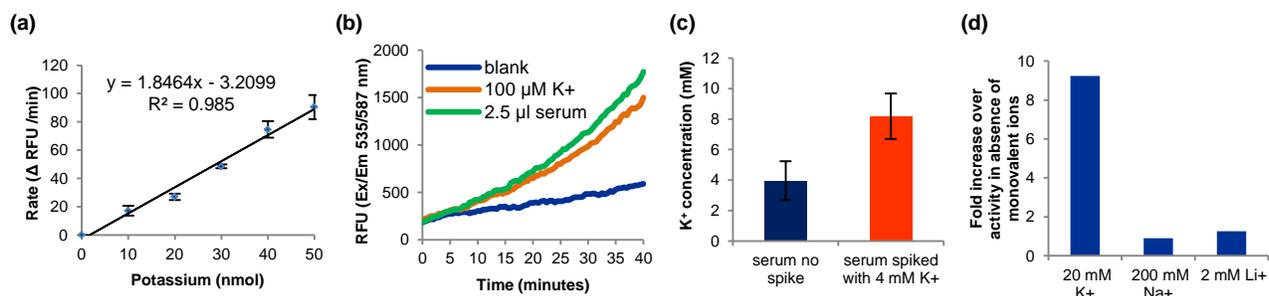


Figure 1: (a) K Standard Curve (b) Reaction kinetics for 100 μ M (10 nmol) K Standard and for 2.5 μ l “off-the-clot” human serum (c) Estimated potassium concentrations in “off-the-clot” human serum. Spiked experiments show $99 \pm 5\%$ recovery. Experiments were run according to kit protocol. (d) Sodium concentrations of up to 10 fold that of potassium do not interfere with the assay.

VIII. RELATED PRODUCTS:

Lithium Assay Kit (Colorimetric) K545
 Sodium Assay Kit (Colorimetric) K391
 Magnesium Colorimetric Assay Kit K385
 Zinc Assay Kit (Fluorometric) (K428)
 Calcium Assay Kit (Fluorometric) K409

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