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Sodium Assay Kit (Colorimetric)

rev 7/15

(Catalog # K391-100; 100 assays; Store at -20°C)

I. Introduction:

Sodium (Na) is one of the most important electrolytes along with chloride, calcium and potassium. Na plays vital roles in the maintenance of plasma volume, pH balance, transmission of nerve impulses, and normal cell functions. Healthy individuals can absorb sodium ingested in food, and kidneys maintain proper sodium balance by excreting its excess in urine. Sodium sources include table salt, milk, meat, shellfish, bread, snack food, etc. Normal Sodium intake has been defined to be between 200-500 mg/day. Patients suffering high blood pressure, hypertension, chronic kidney disease, and people suffering salt sensitivity require restricted low-sodium diets due to those conditions. Hyponatremia (low sodium concentration in blood) can occur in patients with nephrotic syndrome, excessive vomiting and diarrhea, while Hypernatremia (high sodium concentration in blood) is developed in patients suffering from liver diseases, burns, and pregnancy. Traditionally, sodium concentration in clinical settings is determined by potentiometric, gravimetry, photometry, titrimetry and flame atomic emission spectroscopy, but these methods require expensive and complex protocols that need to be performed by trained personnel. BioVision's Sodium Assay Kit offers a simple, two-step colorimetric assay that is based on the requirement of sodium ion as a cofactor for the enzymatic activity of β-Galactosidase (βG). Endogenous mono-, di-, and trivalent ions, ascorbic acid, creatinine, glucose, urea, and bilirubin do not interfere with the assay. The kit can detect Sodium concentration as low as 25 µM in a variety of samples.

Step 1 Apo βG $\xrightarrow{\text{Sodium}}$ βG

Step 2 Substrate $\xrightarrow{\beta G}$ Hydrolysis Products $\xrightarrow{\text{Na Developer}}$ Chromophore (OD 405 nm)

II. Application:

- Estimation of sodium in biological samples

III. Sample Type:

- Biological fluids: serum, urine, saliva, etc.

IV. Kit Contents:

Components	K391-100	Cap Code	Part Number
Na Assay Buffer	25 ml	WM	K391-100-1
Substrate	5 ml	NM	K391-100-2
βG	15 µl	Orange	K391-100-3
DTT (1 M)	0.25 ml	Green	K391-100-4
Na Developer	10 ml	NM	K391-100-5
Na Standard (1.5 M)	1 ml	Yellow	K391-100-6

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **Na Assay Buffer**: Store at -20°C. Bring to room temperature (RT) before use.
- **Substrate:** Store at -20°C, protected from Light. Bring to RT before use. Mix well. If precipitation is observed, sonicate the contents in a water bath sonicator (interval: 2 min). Repeat if necessary. Once opened, use within two months**.**
- **βG:** Store at -20°C. Freeze/thaw should be limited. Once opened, use within two months**.** Keep on ice during use.
- **DTT and Na Developer**: Store at -20°C. Bring to RT before use. Keep both reagents on ice while in use.
- **Na Standard (1.5 M):** Store at -20°C. Bring to RT before use.

VII. Sodium Assay Protocol:

1. **Sample Preparation:** Add DTT to Na Assay Buffer at a final concentration of 10 mM. Make as much as needed. Dilute serum (10-100 times) and urine (25-200 times) using Na Assay Buffer (with DTT). Centrifuge saliva samples at 10,000 x g, 4°C for 10 min. and collect supernatant. Dilute supernatant with Na Assay Buffer (with DTT) (Recommended Dilution Factor: 2-10). Add 1-40 µl of diluted samples into desired well(s) in a 96-well clear plate. Adjust the volume to 40 µl/well with Na Assay Buffer (with DTT).

   Notes:

   - Always prepare fresh Na Assay Buffer with DTT and use within 24 hrs. Keep on ice while in use.
   - Sodium concentration can vary over a wide range. Normal range in humans is 135-145 mM for serum, 40-220 mmol/day (or greater than 20 mM for one-time sample) for urine, and 30-220 mM for saliva. For unknown samples, we strongly recommend diluting the samples, doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.
   - Serum samples should not contain any sodium-salt additives (i.e. Sodium heparin, Sodium EDTA, Sodium Citrate) as they interfere with the results. We recommend using freshly collected serum free of additives or off-the-clot pooled human serum samples.
   - For samples having background, prepare parallel sample well(s) as sample background control. Adjust the volume to 40 µl with Na Assay Buffer (with DTT).

2. **Standard Curve Preparation:** Prepare 7.5 mM Sodium Standard by adding 5 µl of 1.5 M Na Standard to 995 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 7.5 mM Sodium Standard into a series of wells in a 96-well clear plate to generate 0, 15, 30, 45, 60 and 75 nmol of Sodium/well. Adjust the volume to 40 µl/well with Na Assay Buffer (with DTT).
3. **βG Reaction:**
   - **a.** Dilute βG 200 times by adding 1 µl of βG to 199 µl of Na Assay Buffer (with DTT). Make as much as needed. Keep on ice. Add 20 µl of diluted βG into Standard, and sample wells. Incubate plate at 37°C for 10 min., protected from light.
   - **b.** After incubation, add 40 µl of Substrate into each well containing Standards, sample background control, and samples. Mix well. Incubate at 37°C for 30 min. protected from light. After incubation, add 100 µl Na Developer into each well. Mix well.

   Note: Do not store the diluted βG solution.
4. **Measurement**: Measure absorbance (OD 405 nm) in end point mode.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Na Standard Curve. If sample background control reading is significant, then subtract sample background control reading from sample reading. Apply sample's corrected OD to Standard Curve to get B nmol of Sodium in the sample well.

Sample Sodium Concentration (C) = B/V X D nmol/µl or mM

Where: **B** is amount of Sodium in the sample well from Standard Curve (nmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

Sodium Molar Mass: 22.98 g/mol

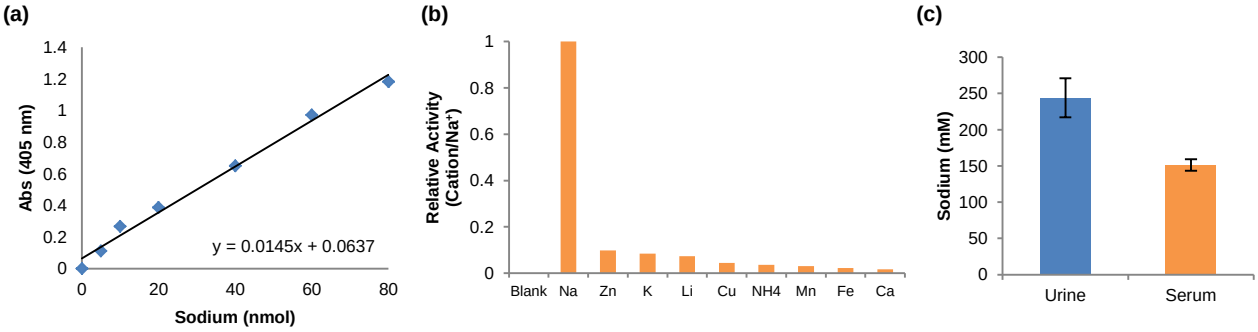

Figure: (a) Sodium Standard Curve. **(b)** Assay Specificity: Sodium, and other mono, di and trivalent cations (15 nmol/0.15 mM each) were tested to evaluate possible interferences. Interferences were found to be less than 10% when data was normalized using Sodium as 100% activity. **(c)** Estimation of sodium in human pooled serum Off-the-Clot (5 µl; 50 times diluted), and human Urine (10 µl; 100 times diluted). Assays were performed following the kit protocol.

VIII. Related Products:

Iron Colorimetric Assay Kit (K390)
Phosphate Fluorometric Assay Kit (K420)
Albumin (Albuminuria) Fluorometric Assay Kit (K550)
Creatinine Colorimetric/Fluorometric Assay Kit (K625)
BCA Protein Assay Kit II (K813)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucose Colorimetric Assay Kit II (K686)
Renin Activity Fluorometric Assay Kit (K800)
Creatine Colorimetric/Fluorometric Assay Kit (K635)
Phosphate Colorimetric Assay Kit (K410)
Zinc Colorimetric Assay Kit (K387)
Urine Albumin-to-Creatinine Ratio (UACR) Assay Kit (K551)
Albumin (BCG) Assay Kit (Colorimetric) (K554)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
Urea Colorimetric Assay Kit (K375)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Renin Inhibitor Screening Kit (Fluorometric) (K799)
Sarcosine Colorimetric/Fluorometric Assay Kit (K636)

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