Sodium Assay Kit (Colorimetric)

Catalog Number MAK247
Storage Temperature –20 °C

**Product Description**

Sodium (Na) ions are one of the most important electrolytes along with chloride, calcium and potassium. Sodium ions play 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 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.

This 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 ion concentrations as low as 25 μM in a variety of samples.

**Components**

The kit is sufficient for 100 assays in 96 well plates.

- **Na Assay Buffer**
  - Catalog Number MAK247A
  - 25 mL

- **Substrate**
  - Catalog Number MAK247B
  - 5 mL

- **βG**
  - Catalog Number MAK247C
  - 15 μL

- **DTT (1 M)**
  - Catalog Number MAK247D
  - 0.25 mL

- **Na Developer**
  - Catalog Number MAK247E
  - 10 mL

- **Na Standard (1.5 M)**
  - Catalog Number MAK247F
  - 1 mL

**Reagents and Equipment Required but Not Provided.**

- 96 well flat-bottom plate – clear plates are recommended for this assay.
- Spectrophotometric multiwell plate reader

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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.

Storage/Stability
The kit is shipped on wet ice and storage at −20 °C, protected from light, is recommended. Allow assay buffer to warm to room temperature before use.

Procedure
All samples and standards should be run in duplicate.

Sample Preparation
Add DTT to Na Assay Buffer at a final concentration of 10 mM. Make as much as needed. Dilute serum (10 to 100-fold) and urine (25 to 200-fold) using Na Assay Buffer (with DTT). Centrifuge saliva samples at 10,000 × g at 4 °C for 10 minutes and collect supernatant. Dilute supernatant with Na Assay Buffer (with DTT) (Recommended Dilution Factor: 2 to 10-fold). 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 hours. 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 mmole/day (or greater than 20 mM for one-time sample) for urine, and 30–220 mM for saliva. For unknown samples, it is strongly recommended to dilute 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. It is recommended to use 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).

Standard Curve Preparation
Use ultrapure water for the preparation of standards. Prepare 7.5 mM Sodium Standard by adding 5 μL of 1.5 M Na Standard to 995 μL of water. Add 0, 2, 4, 6, 8, and 10 μL of 1.5 mM Sodium Standard into a series of wells in a 96 well clear plate to generate 0, 15, 30, 45, 60 and 75 nmole of sodium/well. Adjust the volume to 40 μL/well with Na Assay Buffer (with DTT).

βG Reaction
Dilute βG 200-fold 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, sample background control and sample wells. Incubate plate at 37 °C for 10 minutes, protected from light.

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 minutes, protected from light. After incubation, add 100 μL of Na Developer into each well. Mix well.

Note: Do not store the diluted βG solution.

Measurement
Measure absorbance (OD 405 nm) in end point mode.
Results
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. Compare corrected OD of the sample to the Standard Curve to obtain B (obtain) of sodium in the sample well.

Sodium Concentration (C) = \( \frac{B \times D}{V} \) (nmole/\( \mu \)L or mM)

B = amount of sodium in the sample well from Standard Curve (nmole)
V = sample volume added into the reaction well (\( \mu \)L)
D = sample dilution factor

Sodium Molar Mass: 22.98 g/mole
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<tr>
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<th>Possible Cause</th>
<th>Suggested Solution</th>
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<td>Cold assay buffer</td>
<td>Assay Buffer must be at room temperature</td>
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<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
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<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
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<tr>
<td>Samples with erratic</td>
<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
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<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
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<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if samples will be used multiple times</td>
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<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
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<tr>
<td>Lower/higher readings in</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
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<tr>
<td>samples and standards</td>
<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
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<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Reaction Mix before each use</td>
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<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td>Non-linear standard curve</td>
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<td>Use calibrated pipettes and aliquot correctly</td>
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<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the reaction mix</td>
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<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
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<td>Pipetting errors in the Reaction Mix</td>
<td>Prepare a Reaction Mix whenever possible</td>
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<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the plate well</td>
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<td>Standard stock is at incorrect concentration</td>
<td>Refer to the standard dilution instructions in the Technical Bulletin</td>
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<td>Calculation errors</td>
<td>Recheck calculations after referring to Technical Bulletin</td>
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<td>Substituting reagents from older kits/lots</td>
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<td>Samples measured at incorrect wavelength</td>
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<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
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<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
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