

Product Information

Sodium Assay Kit (Colorimetric)

Catalog Number **MAK247**

Storage Temperature -20°C

TECHNICAL BULLETIN

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 (β G). 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	25 mL
Catalog Number MAK247A	
Substrate	5 mL
Catalog Number MAK247B	
β G	15 μL
Catalog Number MAK247C	
DTT (1 M)	0.25 mL
Catalog Number MAK247D	
Na Developer	10 mL
Catalog Number MAK247E	
Na Standard (1.5 M)	1 mL
Catalog Number MAK247F	

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 $10,000 \times g$ 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.

ResultsCalculation

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.

$$\text{Sodium Concentration (C)} = \frac{B}{V} \times D$$

(nmole/ μ L or mM)

B = amount of sodium in the sample well from Standard Curve (nmole)

V = sample volume added into the reaction well (μ L)

D = sample dilution factor

Sodium Molar Mass: 22.98 g/mole

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Clear plates are recommended for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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