Amplite® Fluorimetric Sodium Ion Quantification Kit

Catalog number: 21325
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21325)
Component A: SoNa™ 520	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Minimize light exposure, Refrigerated (2-8 °C)	1 bottle (20 mL)
Component C: NaCl Standard	Refrigerated (2-8 °C), Minimize light exposure	1 vial
Component D: DMSO	Minimize light exposure, Refrigerated (2-8 °C)	1 vial (100 µL)

OVERVIEW

Quantifying sodium ions is important in various scientific fields and industries, including biochemistry, medicine, environmental analysis, and food science etc. There are several methods commonly used to quantify sodium ions, including flame photometry, ion-selective electrodes (ISE), atomic absorption spectroscopy and fluorescence spectrophotometry. Flame photometry and atomic absorption spectroscopy require the inflammation of the samples. They are tedious to use and require expensive and sophisticated instrumentation. Ion-selective electrodes require large volumes of samples and often have low selectivity. Among all the methods, fluorescence spectrophotometry is the most convenient method for quantifying sodium ions. Fluorescence spectrophotometry involves complexing sodium ions with specific reagents and measuring the resulting fluorescence changes. Amplite® Fluorimetric Sodium Ion Quantification Kit uses our robust sodium ion indicator dye, SoNa™ 520, which exhibit great fluorescence intensity enhancement upon binding to sodium ions. SoNa™ 520 is perhaps the most robust sodium ion indicator with high selectivity. It enables the kit to be useful for the rapid determination of sodium concentrations in a variety of samples compared to the other commercial sodium ion assays. This microplate-based assay kit requires extremely small amount of sample, it is particularly suitable for the determination of sodium ion concentration in microvolume format.

AT A GLANCE

Protocol Summary

1. Add 50 µL NaCl Standards or test samples
2. Add 50 µL SoNa™ 520 working solution.
3. Incubate at RT for 5-10 minutes
4. Monitor the fluorescence at Ex/Em=490/525 nm

Important

The following protocol is an example for quantifying sodium content using SoNa™ 520. Allow all the components to warm to room temperature before opening. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	515 nm
Emission	525 nm
Excitation	490 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Prepare SoNa™ 520 stock solution

1. Add 100 µL of DMSO (Component D) into SoNa™ 520 vial (Component A).

   Note: Make a single unused SoNa™ 520 stock solution aliquot and store at ≤ -20 ° C. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21325

NaCl Standard
Add 300 µL of distilled water to the NaCl Standard vial (Component C) to make a 250 mM standard stock solution. Next, dilute this 250 mM stock solution using Assay Buffer (Component B) to make a 40 mM (SS1). Then perform 1:2 serial dilutions to get serially diluted NaCl standard (SS2 – SS7).

PREPARATION OF WORKING SOLUTION

Prepare SoNa™ 520 working solution

1. Add 100 µL of SoNa™ 520 (Component A) into 5 mL of Assay Buffer (Component B). Protect the working solution from light by covering it with foil or placing it in the dark.

   Note: For best results, this solution should be used within a few hours of its preparation.

   Note: 5 mL of working solution is enough for 100 tests.

SAMPLE EXPERIMENTAL PROTOCOL

Important

The following protocol only provides a guideline and should be modified according to your specific needs.

Table 1. Layout of NaCl standards and test samples in a solid black 96-well microplate.

SS=NaCl Standards (SS1 - SS7, 40 to 0.625 mM, 2X dilutions); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
SS1	SS1	...	...
SS2	SS2	...	...
SS3	SS3	...	...
SS4	SS4	...	...
SS5	SS5	...	...
SS6	SS6	...	...
SS7	SS7	...	...

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SS1-SS7	50 µL	Serial dilutions (40 to 0.625 mM)
BL	50 µL	Assay Buffer
TS	50 µL	Sample

Protocol

1. Prepare NaCl standards (SS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of SoNa™ 520 working solution to each well of NaCl standards, blank control, and test samples to make the assay volume of 100 µL/well. For a 384-well plate, add 25 µL into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/525 nm (cut off at 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

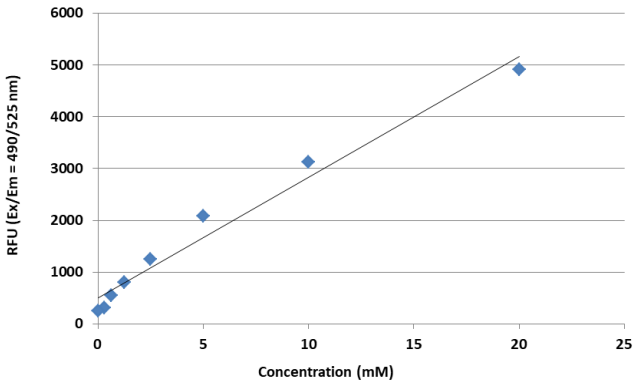

Figure 1. Sodium dose response was measured with Amplite® Fluorimetric Sodium Ion Kit in a 96-well solid black plate.

DISCLAIMER

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