Amplite™ Total Sulfide Quantification Kit

Catalog number: 21300
Unit size: 100 Tests

Component	Storage	Amount
Component A: Sulfide Green™	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Sulfide Assay Buffer	Freeze (< -15 °C)	1 bottle (12.5 mL)
Component C: Sulfide Standard (50 mM)	Freeze (< -15 °C)	1 vial (0.2 mL)
Component D: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)

OVERVIEW

Hydrogen sulfide is not usually a health risk at concentrations present in household water. Water containing hydrogen sulfide, commonly called sulfur water, has a distinctive "rotten egg" odor, which may be especially noticeable when running hot water. Such water can discolor coffee, tea and other beverages, and alter the appearance and taste of cooked foods. However, hydrogen sulfide dissolved in water can corrode plumbing metals, such as iron, steel, copper and brass and exposed metal parts in washing machines and other water-using appliances. The corrosion of iron and steel from hydrogen sulfide forms ferrous sulfide or "black water" which can darken silverware and discolor copper and brass utensils. Hydrogen sulfide can also interfere with the effectiveness of water softeners. High sulfide concentration is poisonous. Amplite™ Sulfide Quantification Kit provides a robust method for detecting sulfide anion in solution. It uses Sulfide Green™, a selective and sensitive green sulfide probe that can be easily monitored with a fluorescence microplate reader (Ex/Em = 490/530 nm). Upon reaction with sulfide anion, Sulfide Green generates strong green fluorescence that is proportional to sulfide concentration. Amplite™ Fluorimetric Sulfide Anion Quantitation Kit can be performed in a convenient 96-well or 384-well microplate format and easily adapted to automation with no separation steps required. The assay can be completed within 30 minutes.

AT A GLANCE

Protocol summary

1. Add Sulfide Standards or test samples (50 µL)
2. Add Sulfide Green™ working solution (50 µL)
3. Incubate at room temperature for 30-60 minutes
4. Monitor fluorescence intensity at Ex/Em = 490/530 nm (Cut off: 515nm)

Important
Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	530 nm
Cutoff	515 nm
Recommended plate	Solid black
Instrument specification(s)	Top read mode

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.

Sulfide Green™ stock solution (200X)
Add 30 µL of DMSO (Component D) into the vial of Sulfide Green™ (Component A) to make 200 X Sulfide Green™ stock solution.

Note Unused stock can be stored into smaller aliquotes at -20 °C to minimize frquent freeze and thaw.

PREPARATION OF STANDARD SOLUTION

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

Sulfide Standard
Add 100 µL of 50 mM Sulfide Standard (Component C) to 400 µL Sulfide Assay Buffer (Component B) to generate 10000 µM Sulfide Standard solution. Note: If the sulfide concentration is in the high dose range (100 uM to 10mM), please use the HIGH range standard curve dilution, and if the sulfide concentration is in the low dose range (1 uM to 10uM), please use the LOW range standard curve dilution. (For details see Preparation of Working Solution #2)

PREPARATION OF WORKING SOLUTION

1. Sulfide Green™ working solution
Add 25 µL of Sulfide Green™ stock solution into 5 mL of Assay Buffer (Component B) to make a total volume of 5.025 mL.

Note Protect the working solution from light by covering it with foil or placing it in the dark.

2. Sulfide Standard solution
High range standard curve dilution: Take 10,000 µM Standard solution (SS1) to perform 1:3 serial dilutions by Sulfide Assay Buffer (Component B) to get serially diluted Sulfide Standards ranging from 100 to 10000 µM (SS2- SS7).
Low range standard curve dilution: Add 10 µL of 10000 uM Sulfide solution to 990 µL Sulfide Assay Buffer (Component B) to generate 100 µM Sulfide Standard solution (SS1). Take 100 µM standard solution to perform 1:3 serial dilutions by Sulfide Assay Buffer (Component B) to get serially diluted Sulfide Standards ranging from 1 to 100 µM (SS2- SS7).

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Sulfide Standards and test samples in a solid black 96-well microplate. SS= Sulfide Standards (SS1-SS7, 1000 - 1.5 µM); 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	Reagents
SS1-SS7	50 µL	Serial dilutions (Low range: 1 - 100 µM) Serial dilutions (Low range: 100 - 10000 µM)
BL	50 µL	Assay Buffer
TS	50 µL	Test Samples

1. Prepare Sulfide 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.

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2. Add 50 µL of working solution to each well of Sulfide Standard, blank control, and test samples to make the total sulfide assay volume of 100 µL/well. For a 384-wellplate, add 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 30-60 minutes, protected from light.

4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 490/530 nm (cut off at 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Sulfide Concentration samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

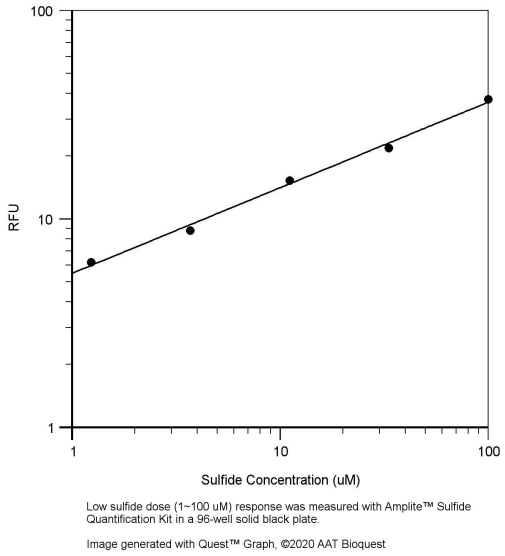

Figure 1. Low sulfide dose (1~100 uM) response was measured with Amplite™ Sulfide Quantification Kit in a 96-well solid black plate.

DISCLAIMER

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