Amplite™ Fluorimetric Mercuric Ion Quantitation Kit

Catalog number: 19005
Unit size: 100 tests

Component	Storage	Amount
Component A: Mercury Lite™ 590	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Mercuric ion is an essential metal ion that plays an important role in a number of biological processes. Mercury ion is considered to be one of the most hazardous pollutants and highly dangerous materials. The high toxicity of Hg2+ is caused by its high affinity to the thiol groups in biological ligands such as proteins, DNA, and enzymes. When Hg2+ is absorbed in the human body from the environment, it induces aberrations in microtubules, ion channels, and mitochondria presumably and significant damage to the kidneys, heart, brain, stomach, intestines, central nervous system and immune systems. In addition, mercury accumulates through food chains or atmosphere in the ecological system, and has a relatively long atmospheric residence time because of its non-biodegradation. It has been intensively studies for a variety reasons, in particular, its environmental impacts and biological complications. However, there are very few commercial products that were developed for rapid detection mercuric ion. The highly selective and sensitive detection of mercury is of toxicological and environmental importance. Amplite™ Fluorimetric Mercuric Ion Quantitation Kit offers a robust fluorescence-based assay for measuring mercury ion (Hg2+) with high selectivity. Mercury Lite™ 590 itself is nearly non-fluorescent, but generates more than 500-fold fluorescence enhancement upon binding Hg2+ ion. The fluorescence signal can be measured with a fluorescence microplate reader. With this kit, we were able to detect as low as 8 µM Hg2+ in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare mercury working solution (50 µL)
2. Add mercury (II) standard or test samples (50 µL)
3. Incubate at room temperature for 20 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

Important To achieve the best results, it's strongly recommended to use the black plates. Thaw kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 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.

1. Mercury Lite™ 590 stock solution (200X):
Add 25 µL of DMSO into the vial of Mercury Lite™ 590 (Component A) and mix them well. Keep from light.

Note Make single use aliquots, and store unused 200X Mercury Lite™ 590 stock solution at -20ºC, avoid light and repeat freeze-thaw cycles.

2. Mercury (II) standard stock solution (not provided):
We used Mercury (II) Perchlorate hydrate (Sigma 529656, CAS#304656-34-6) as the mercury (II) standard. The stock solution of mercury (II) was prepared at the concentration of 1 mM in ddH_2O.

PREPARATION OF STANDARD SOLUTION

Mercury (II) standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/19005

Perform 1:2 serial dilutions using ddH_2O to get approximately 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µM serially diluted mercury (II) standards (M7 - M1).

PREPARATION OF WORKING SOLUTION

Add 25 µL of Mercury Lite™ 590 stock solution into 5 mL of Assay Buffer (Component B) and mix them well (Component A+B).

Note This mercury working solution is enough for one 96-well plate. It is not stable, use it promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of mercury (II) standards and test samples in a solid black 96-well microplate. M= Mercury (II) Standards (M1 - M7, 7.8 to 500 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
M1	M1	...	...
M2	M2	...	...
M3	M3		
M4	M4		
M5	M5		
M6	M6		
M7	M6		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
M1 - M7	50 µL	Serial Dilutions (7.8 to 500 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Prepare and add mercury (II) standards (M), 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 mercury working solution to each well of mercury (II) standard, blank control, and test samples to make the total mercury assay volume of 100 µL/well. For a 384-well plate, add 25 µL of mercury working solution into each

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well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 20 - 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm, cutoff 570 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 Mercury II 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

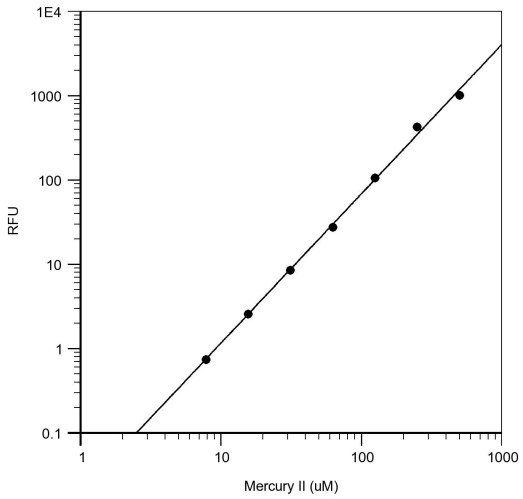

Figure 1. Hg2+ was measured with the Amplite™ Fluorimetric Mercuric Ion Quantitation Kit (Cat#19005) in a 96-well solid black plate using a Gemini microplate reader (Molecular Devices). As low as 8 µM mercury (II) perchlorate was detected with 30 minutes incubation.

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