Amplite™ Fluorimetric Mercuric Ion Quantitation Kit

Orange Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 19005 (100 Tests)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

Mercury ion is considered to be one of the most hazardous pollutants and highly dangerous materials. The high toxicity of Hg^{2+} is caused by its high affinity to the thiol groups in biological ligands such as proteins, DNA, and enzymes. When Hg^{2+} 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. Therefore, highly selective and sensitive detection of mercury is of toxicological and environmental importance.

Amplite™ Fluorimetric Mercuric Ion Quantitation Kit offers a fluorescence-based assay for measuring mercury ion (Hg^{2+}) with high selectivity. Mercury Lite™ 590 itself is nearly non-fluorescent， but generates more than 500-fold fluorescence enhancement upon binding Hg^{2+} ion. The fluorescence signal can be measured with a fluorescence microplate reader at Ex/Em= 540/590 nm. With this kit, we were able to detect as low as 8 µM Hg^{2+} in a 100 µL reaction volume.

Kit Components

Components	Amount
Component A: Mercury Lite™ 590	1 vial
Component B: Assay Buffer	1 bottle (10 mL)
Component C: DMSO	1 vial (100 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare mercury assay mixture (50 µL) → Add mercury (II) standard or test samples (50 µL) → Incubate at room temperature for 20-30 min → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Notes: 1. To achieve the best results, it's strongly recommended to use the black plates.

2. Thaw kit components at room temperature before starting the experiment.

1. Prepare Mercury Lite™ 590 stock solution (200X):

Add 25 µL of DMSO into the vial of Mercury Lite™ 590 (**Component A**) to make 200X stock solution.

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

2. Prepare mercury assay mixture:

Add 25 µL of Mercury Lite™ 590 stock solution (from Step 1) into 5 mL of Assay Buffer (**Component B**), and mix well to make mercury assay mixture (**Component A+B).**

Note 1: This mercury assay mixture is enough for one 96-well plate. It is not stable, use it promptly.

Note 2: One can divide unused mercury assay mixture into single use aliquots and stored at -20°C.

3. Prepare 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 ddH2O. The stock solution should be divided into single use aliquots and stored at -20°C.

4. Prepare serial dilutions of mercury (II) standard (0 to 500 µM):

4.1 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.

4.2 Add serial dilutions of mercury (II) standard and Hg^{2+} containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

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Table1: Layout of mercury (II) standards and test samples in a solid black 96-well microplate

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

Note: M= Mercury (II) Standards, BL=Blank Control, TS=Test Samples.

Table2: Reagent composition for each well

Mercury (II) Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer : 50 µL	50 µL

**Note: Add the serially diluted mercury (II) standards from approximately 8 µM to 500 µM (or 1.6 ppm to 100 ppm) into wells from M1 to M7 in duplicate.*

5. Run mercury assay:

5.1 Add 50 µL of mercury assay mixture (from Step 2) to each well of mercury (II) standard, blank control, and test samples (see Step 4.2) to make the total volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of mercury assay mixture into each well.

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

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

Data Analysis

The fluorescence reading in blank wells (with assay buffer and mercury assay mixture only) is used as a control, and is subtracted from the values of those wells with the Hg^{2+} standards and test samples. A Hg^{2+}standard curve is shown in Figure 1.

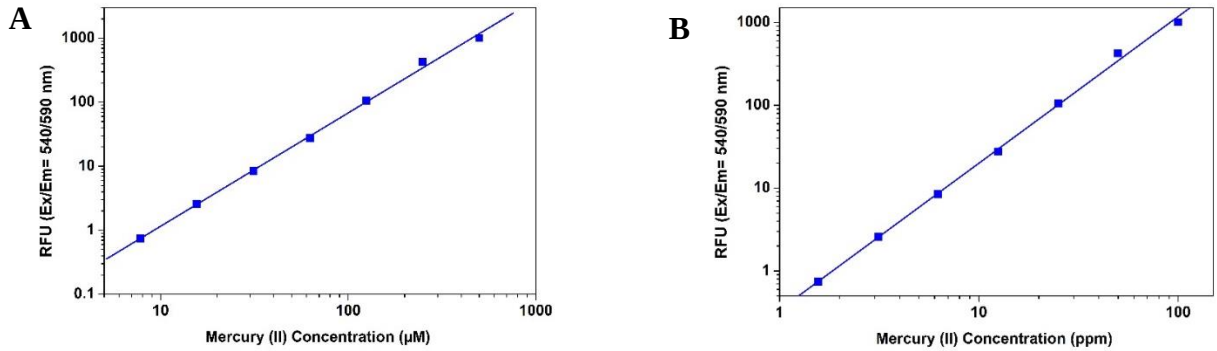

Figure 1. Hg^{2+} was measured with the Amplite™ Fluorimetric Mercuric Ion Quantitation Kit (Cat#19005) in a 96-well solid black plate using a Gemini microplate reader (Molecular Devices). As low as 8 µM (A) or 1.6 ppm (B) mercury (II) perchlorate was detected with 30 minutes incubation. (*Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point).*

Reference

1. Ozuah PO. (2000) Mercury poisoning. Current Problems in Pediatrics 30(3): 91-99.
2. Schroeder WH and Munthe J. (1998) Atmospheric mercury—an overview. Atmospheric Environment 32(5): 809-822.
3. Hylandera LD and Goodsite ME. (2006) Environmental costs of mercury pollution. Science of the Total Environment 368(1): 352-370.