Version 1 Last updated 27 March 2018

ab233467
Mercury Assay Kit

For the measurement of mercury ion (Hg^{2+}) with high selectivity.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Mercury Assay Kit (ab233467) offers a fluorescence-based assay for measuring mercury ion (Hg^{2+}) with high selectivity. Mercury sensor 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.

Prepare mercury assay mixture.

Add mercury (II) standard or test samples.

Incubate at room temperature for 20-30 minutes.

Monitor fluorescence intensity at Ex/Em = 540/590 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)
Mercury sensor 590	1 vial	-20°C
Assay Buffer	10 mL	-20°C
DMSO	100 µL	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader capable of reading at Ex/Em= 540/590 nm.
- Mercury (II) Perchlorate hydrate (CAS#304656-34-6).
- ddH_2O.
- Solid black 96-well or 384-well plate

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Mercury sensor 590 stock solution (200X)

Add 25 µL of DMSO into the vial of Mercury sensor 590 to make 200X stock solution.

Δ Note Make single use aliquots, and store unused 200 X Mercury sensor 590 stock solution at -20°C, avoid light and repeat freeze-thaw cycles.

5.2 Mercury assay mixture

Add 25 µL of Mercury sensor 590 stock solution (from Step 5.1) into 5 mL of Assay Buffer, and mix well to make mercury assay mixture.

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

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

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. We used Mercury (II) Perchlorate hydrate (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. The stock solution should be divided into single use aliquots and stored at -20°C.
2. 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.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Mercury Assay:

1. Add 50 µL serial dilutions of mercury (II) standard and Hg^{2+} containing test samples into a solid black 96-well microplate in duplicate.
2. Add 50 µL Assay Buffer as a Blank Control into a solid black 96-well microplate in duplicate.
3. Add 50 µL of mercury assay mixture (from Reagent Preparation Step 5.2) to each well of mercury (II) standard, blank control, and test samples to make the total volume of 100 µL/well.
   Δ Note For a 384-well plate, add 25 µL of sample and 25 µL of mercury assay mixture into each well.
4. Incubate the reaction at room temperature for 20-30 minutes, protected from light.
5. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm, cutoff 570 nm.

8. 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 Hg2+ standards and test samples.

9. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

10. Typical Data

Data provided **for demonstration purposes only**.

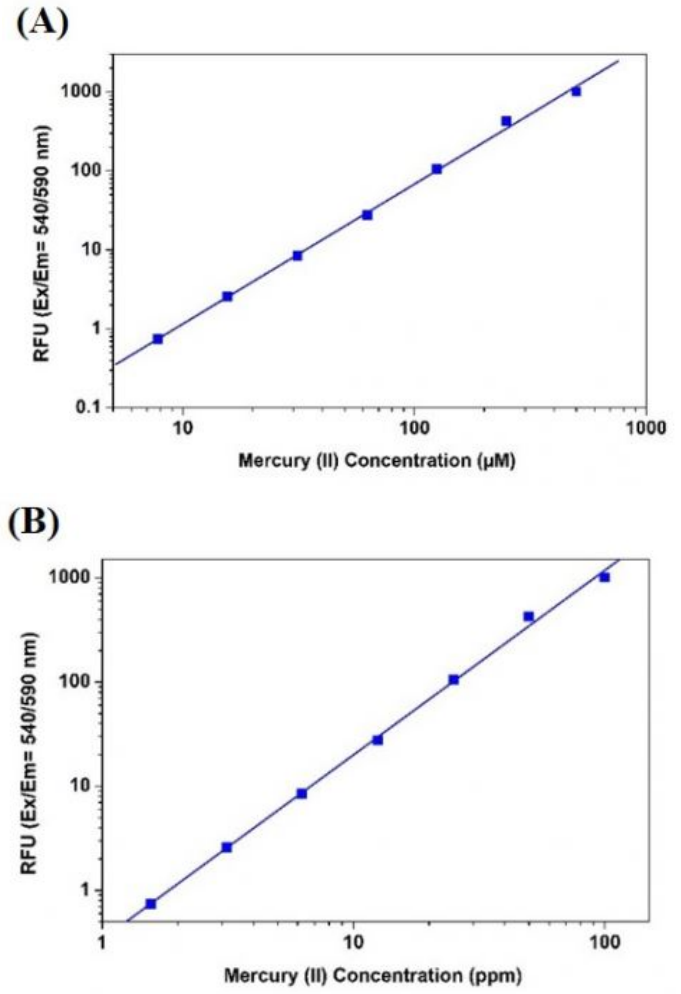

Figure 1. Hg^{2+} was measured with ab233467 in a 96-well solid black plate using a Gemini microplate reader (Molecular Devices). As low as 8 µM (A) or 1.6 ppm (B) mercury (II) perchlorate was detected with 30 minutes incubation. (Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point)..

11. Notes

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