

## Water Mercury Ion (Hg<sup>2+</sup>) Content Assay Kit

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer

**Cat Number:** BC2820

**Size:** 50T/48S

### Components:

**Reagent I:** Powder×2. Store at 2-8°C. Add 1 mL of distilled water to one bottle before use. The unused reagent can be stored at -20°C for one week. Avoid repeated freezing and thawing.

**Reagent II:** Liquid 5 mL×1. Store at 2-8°C.

**Reagent III:** Liquid 12 mL×1. Store at 2-8°C.

**Reagent IV:** Powder×1. Store at 2-8°C. Dissolve with 5 mL of distilled water before use. The unused reagent can be stored at 2-8°C for two weeks.

**Reagent V:** Powder×2. Store at 2-8°C and protect from light. Dissolve one bottle with 50 mL of chloroform (**self-provided**) before use. The unused reagent can be stored at 2-8°C for one week.

**Reagent VI:** Liquid 20 mL×1. Store at 2-8°C.

**Standard Solution:** Liquid 1 mL×1, 4000 nmol/mL Hg<sup>2+</sup> standard solution. Store at room temperature. Dilute 400 times with distilled water to form a standard solution of 10 nmol/mL before use. The reagent should be prepared just before use.

### Product Description:

Hg<sup>2+</sup> is an important toxic heavy metal ion in water, which easily absorbed and accumulated by organisms and can be further transmitted through the food chain, causing damage. Minamata disease is a kind of typical mercury poisoning.

After digestion, Hg<sup>2+</sup> can form one orange complex with Dithizone in acid environment, which can be dissolved in chloroform and has a maximum absorption peak at 490 nm. In this kit, the content of Hg<sup>2+</sup> is quantified by measuring the color development at 490 nm.

### Reagents and Equipment Required but Not Provided.

Spectrophotometer, centrifuge, adjusted transferpette, 1 mL glass cuvette, concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 98%), concentrated nitric acid (HNO<sub>3</sub>, 98%), chloroform, distilled Water.

### Procedure:

#### I. Sample preparation

Add 7 mL of concentrated nitric acid immediately after every 1000 mL of water sample is collected. Adjust the pH ≤ 1. If the water sample cannot be measured immediately after sampling, add 4 mL or more of Reagent II to each liter of sample to make it lasting pale red.

#### II. Detection:

1. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 490 nm, set zero with

chloroform.

2. Add reagents with the following list to 5 mL EP tubes:

Reagent ( $\mu\text{L}$ )	Test Tube ( $A_T$ )	Standard Tube ( $A_S$ )	Blank Tube ( $A_B$ )
Water sample	1000	-	-
Standard solution	-	1000	-
Distilled water	-	-	1000
Concentrated sulfuric acid	40	40	40
Concentrated nitric acid	10	10	10
Reagent I	32	32	32
Reagent II	60	60	60
Seal with parafilm, mix thoroughly and shock 2 minutes. Digest in $95^\circ\text{C}$ water bath for 2 hours, then cool to about $40^\circ\text{C}$ .			
Reagent III	200	200	200
Shake until the solution in the EP tube is clear and transparent. Open the lid and leave for 10 minutes. Shake several times during standing to allow the gas escape.			
Reagent IV	80	80	80
Reagent V	1000	1000	1000
Fully shake for 2 minutes after capping, let stand for 10 minutes. Take $900\mu\text{L}$ of the organic phase in the lower layer into 1.5mL EP tubes.			
Reagent VI	400	400	400
Fully shake to make the organic phase without green. After standing and delaminating, absorb the organic phase and measure the absorbance at 490 nm. Recorded as $A_T$ , $A_S$ , $A_B$ . $\Delta A_T = A_T - A_S$ , $\Delta A_B = A_B - A_S$ . The standard tube and blank tube only need to be measured 1-2 times.			

### III. Calculations

$$\text{Hg}^{2+} \text{ content (nmol/mL)} = C_s \times \Delta A_T \div \Delta A_S = 10 \times \Delta A_T \div \Delta A_S$$

$C_s$ : Content of  $\text{Hg}^{2+}$  standard solution, 10 nmol/mL.

#### Note:

- 1000  $\mu\text{g/L}$  copper ion, 20  $\mu\text{g/L}$  silver ion, 10  $\mu\text{g/L}$  gold ion, 5  $\mu\text{g/L}$  platinum ion in water sample without interference.
- Pay attention to safety during measurement, wear masks and gloves to avoid inhalation or exposure to toxic and dangerous reagents.
- When the absorbance is greater than 1, please dilute the serum to appropriate concentration with distilled water. Multiply by the corresponding dilution factor in the formula.
- Water with less suspended matter and/or organic matter can shorten the heating time to 1 hour, and clean water without suspended matter can shorten the heating time to 30 minutes.

5. If the upper solution of sample tube becomes transparent during digestion, Reagent II can be added appropriately to keep the sample tube pink or black purple.
6. If the added Reagent III is not enough to make the sample tube clear, the amount of Reagent III can be increased to make the sample tube clear.
7. If the lower organic phase still appears green after Reagent VI is added, the amount of Reagent VI can be increased to make the organic phase transition of the lower layer shallow.

**Related Products:**

BC2830/BC2835	Water Chromium (Cr <sup>6+</sup> ) Content Assay Kit
BC2850/BC2855	Total Phosphorus Content Assay Kit
BC4350/BC4355	Tissue Iron Content Assay Kit
BC4380/BC4385	Blood Ammonia Content Assay Kit