TECHNICAL MANUAL

Lead Ion (Pb2+) Fluorometric Assay Kit

- **SKU CODE:** MAES0537
- **SIZE:** 96 Tests
- **PRODUCT TYPE:** Fluorometric
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Key Features

Detection Range: 0.1-5.0 mg/L

Measuring Instrument: Fluorescence Microplate Reader (Ex/Em=270 nm/480 nm)

2. Intended use

This kit is designed to measure lead ion (Pb2+) content in water samples.

3. Detection principle

Lead ions (Pb2+) form complexes with chromogenic reagents in the reaction system. When excited by 270 nm ultraviolet light, blue fluorescence is emitted at 480 nm. The fluorescence intensity exhibits a linear relationship with

4. Kit components & storage

Store all reagents according to the storage conditions specified in the table below. Reagents from different kits should not be mixed. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Matrix Solution	27.5 mL × 1 vial	55 mL × 1 vial	2-8°C, 12 months
Reagent 2	500 mg/L Lead Standard Solution	0.2 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months
Reagent 3	Iodine Solution	5.5 mL × 1 vial	11 mL × 1 vial	2-8°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=270 nm/480 nm), electric hot plate

Reagents

Concentrated nitric acid, lead-free double distilled water

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.

2. Preparation of 25 mg/L lead standard solution:

Prepare sufficient lead standard solution before testing. For example, to prepare 1000 µL of 25 mg/L lead standard solution, mix 50 µL of 500 mg/L lead standard solution with 950 µL of matrix solution and mix well. The 25 mg/L lead standard solution should be prepared fresh, protected from light, and used within 8 hours.

3. Preparation of standard curve:

Always prepare fresh standard solutions. Discard working standard dilutions after use.

Dilute 25 mg/L lead standard solution with matrix solution to create a serial dilution. The recommended dilution gradient is: 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 mg/L. Reference preparation is as follows:

Item	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Concentration (mg/L)	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
25 mg/L Lead standard (µL)	0	20	40	60	80	120	160	200
Matrix solution (µL)	1000	980	960	940	920	880	840	800

7. Sample preparation

Water sample

If the water sample is free of suspended solids and relatively clean, take a small amount of water sample and dilute it 10-fold with matrix solution for direct determination.

If the water sample contains significant suspended solids and organic matter, add 1 mL of concentrated nitric acid and 0.1 mL of iodine solution per 100 mL of sample. Heat on an electric hot plate to 95-100°C and gently boil for digestion for 10 minutes. After cooling, adjust the volume to 100 mL with lead-free double distilled water. Take a small amount of the treated water sample and dilute it 10-fold with matrix solution for direct determination.

Dilution of sample

Note: Use matrix solution as the diluent. For dilution of other sample types, please perform a pretest to confirm the appropriate dilution factor.

8. The key points of the assay

When adding concentrated nitric acid to the water sample, heat carefully and monitor the remaining volume of the water sample.

2. If the measured value is near the detection limit, the water sample should be heated and concentrated before measurement. In this case, the dilution factor in the calculation formula should be modified accordingly.

9. Operating steps

1. Standard wells: Add 200 µL of standard solution at different concentrations to the corresponding wells. Sample wells: Add 200 µL of sample to the wells.
2. Incubate in the refrigerator at 2-8°C for 20 minutes. Measure fluorescence at excitation wavelength 270 nm and emission wavelength 480 nm, recorded as F.

10. Calculation

The standard curve

1. Average the duplicate readings for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings. This is the absolute fluorescence value.
3. Plot the standard curve using the absolute fluorescence values of standards and corresponding concentrations as y-axis and x-axis

y = ax + b

respectively. Create the standard curve (y = ax + b) using graph software (or Excel).

Water samples

Pb2+ content (mg/L) = (ΔF - b)/a × 10* × f

[Note]

ΔF: ΔF = Fsample – Fblank.

10*: Dilution factor of sample during sample preparation.

f: Dilution factor of sample before testing.

11. Appendix I Performance Characteristics

Intra-assay Precision

Three water samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	1.25	2.50	3.75
%CV	1.5	0.8	1.2

Inter-assay Precision

Three water samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	1.25	2.50	3.75
%CV	6.0	4.9	4.8

Recovery

Three samples of high, medium, and low concentrations were tested in parallel (6 replicates each) to determine recovery rates, yielding an average recovery rate of 105%.

Sample 1	Sample 2	Sample 3
1.25	2.50	3.75

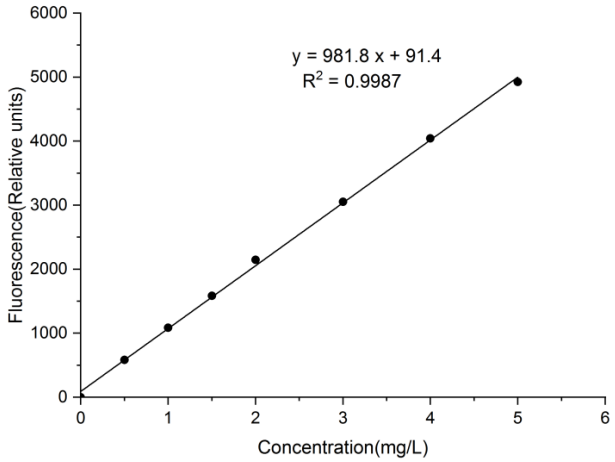
Sample 1	Sample 2	Sample 3
1.33	2.60	3.94
106	104	105

Sensitivity

The analytical sensitivity of the assay is 0.1 mg/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

The fluorescence values of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects). Therefore, the standard curve and data provided below are for reference only:



Concentration (mg/L)	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
F value	355	929	1433	1919	2536	3486	4439	5190
	385	976	1477	1991	2498	3356	4389	5294
Average F value	370	953	1455	1955	2517	3421	4414	5242

Concentration (mg/L)	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Absoluted F value	0	583	1085	1585	2147	3051	4044	4872

12. Statement

1. Assay Genie is not responsible for any problems or legal liabilities arising from using this kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate instruments before experiments. Follow the instructions strictly during experiments.

3. Use appropriate protection methods by wearing lab coats and latex gloves.

4. If the substance concentration is not within the detection range, perform additional dilution or concentration of the sample.

5. A pre-test is recommended if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent conditions, operations, environment, and other factors. Assay Genie guarantees only the quality of the kits and is NOT responsible for sample consumption caused by kit usage. Calculate the

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.