（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F106

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=270 nm/480 nm)

Detection range：0.1-5.0 mg/L

Elabscience® Lead Ion (Pb^{2+}) Fluorometric Assay Kit

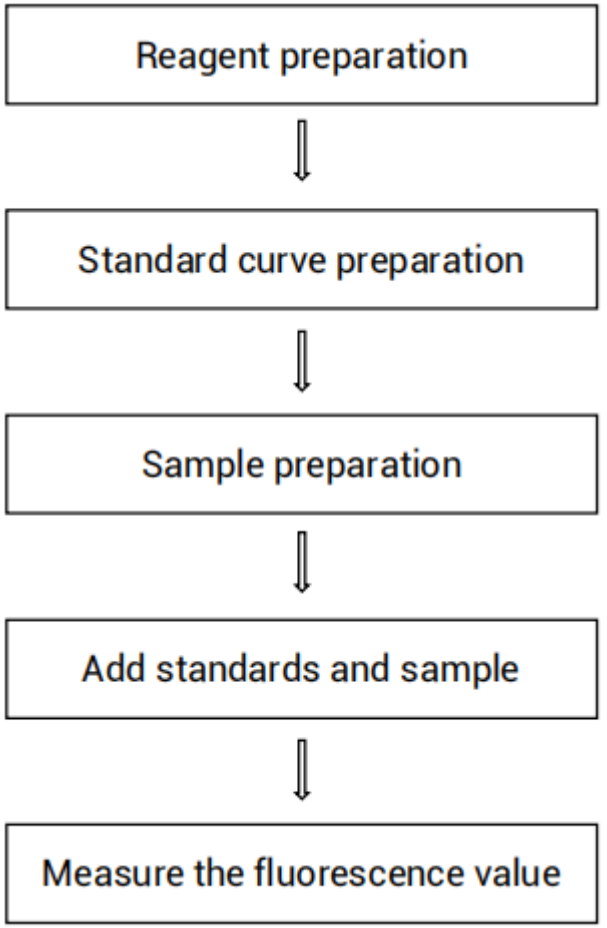
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure lead ion (Pb^{2+}) content in water.

Detection principle

Lead ions (Pb^{2+}) 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 has a linear relationship with the concentration of lead ions within a certain range.

Kit components & storage

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		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of 25 mg/L lead standard solution:

Before testing, please prepare sufficient lead standard solution. For example, prepare 1000 μL of 25 mg/L lead standard solution (mix well 50 μL of 500 mg/L lead standard solution and 950 μL of matrix solution). The 25 mg/L lead standard solution should be prepared on spot protected from light and used up within 8 h.

③ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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

Sample preparation

① 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 times with matrix solution to determine it directly.

If the water sample contains a large amount of suspended solids and organic matter, add 1 mL of concentrated nitric acid and 0.1 mL of iodine solution for every 100 mL. Heat it on an electric hot plate to 95-100°C and gently boil for digestion for 10 min. After cooling, make up the volume to 100 mL with lead-free double distilled water. Take a small amount of water sample and dilute it 10 times with matrix solution to determine it directly.

② Dilution of sample

Note: The diluent is matrix solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① After adding concentrated nitric acid to the water sample, heat it carefully and pay attention to the remaining volume of the water sample.

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

Operating steps

① Standard well: add 200 μL of standard solution with different concentrations into the corresponding wells.
Sample well: add 200 μL of sample into the wells.

② Place in the refrigerator at 2-8°C for 20 min. Measure the fluorescence at the excitation wavelength of 270 nm and the emission wavelength of 480 nm, recorded as F.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.
3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Water samples:

$$\underset{(mg/L)}{Pb^{2+}\ content} = \frac{\Delta F - b}{a} \times 10* \times f$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{blank}$.

10*: Dilution factor of sample during sample preparation.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

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

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc(mg/L)	1.25	2.50	3.75
Observed Conc(mg/L)	1.33	2.60	3.94
Recovery rate (%)	106	104	105

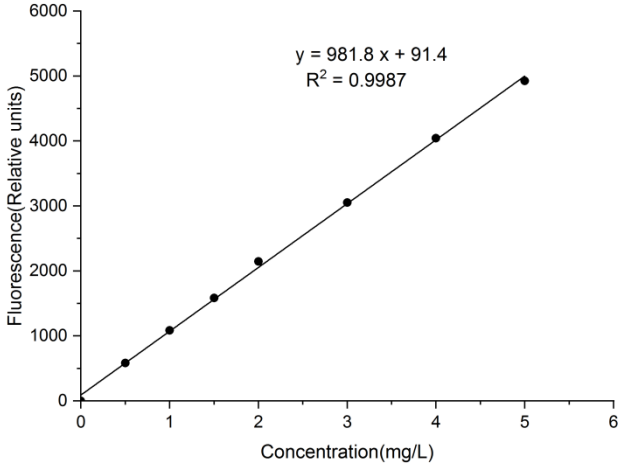
Sensitivity

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

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below 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
Absoluted F value	0	583	1085	1585	2147	3051	4044	4872



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.