Amplite™ Fluorimetric Lead (II) Ion Quantitation Kit

Catalog number: 19007
Unit size: 200 Tests

Component	Storage	Amount
Component A: Lead Green	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Lead (II) Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component C: Lead Standard (100 mM)	Freeze (<-15 °C)	1 vial (0.2 mL)
Component D: DMSO	Freeze (<-15 °C)	1 vial (100 uL)

OVERVIEW

Lead is a highly poisonous metal to human beings, especially young children. Lead interferes with cell signaling and gene expression, causing severe damages to the brain, liver, kidney, and even death. Because of its long history in mining and smelting, as well as widespread use in battery, paint and gasoline, lead pollution in soil and groundwater has been one of the most serious environmental problems. Amplite™ Fluorimetric Lead (II) Ion Quantitation Kit provides a robust method for detecting lead (II) ion in solution. It uses Lead Green™, a selective and sensitive green fluorescence probe that can be easily monitored with a fluorescence microplate reader (Ex/Em = 490/530 nm). The Amplite™ Fluorimetric Lead (II) Ion Quantitation Kit can be performed in a convenient 96-well or 384-well microplate format and easily adapted to automation with no separation steps required. The assay can be completed within 30 minutes. With the Amplite™ Fluorimetric Lead (II) Ion Quantitation Kit, as little as 4μM lead (II) ion was detected.

AT A GLANCE

Protocol summary

1. Prepare and add lead standards or test samples (50 uL)
2. Prepare and add Lead Green working solution (50 uL)
3. Incubate at room temperature for 10-30 minutes
4. Monitor fluorescence intensity at Ex/Em = 490/530 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	530 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Lead Green stock solution (200X):
Add 50 uL of DMSO (Component D) into the vial of Lead Green (Component A) to make Lead Green stock solution (200X).

Note Store the unused Lead Green stock solution at -20 °C in single use aliquots.

PREPARATION OF STANDARD SOLUTION

Lead standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/19007

Add 10 uL of 100 mM Lead (II) Standard solution (Component C) to 990 uL H_2O to generate 1 mM Lead standard solution. Take 1 mM Lead (II) standard solution to perform 1:3 serial dilutions by H_2O to get serially diluted Lead (II) standards ranging from 0 to 1 mM.

PREPARATION OF WORKING SOLUTION

Lead Green working solution:
Add 25 uL Lead Green stock solution (200X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.025 mL.

Note Keep away from Light.

Note Lead Green working solution should be used promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of lead standards and test samples in a solid black 96-well microplate. LS = Lead standard (LS1-LS7, 1000 to 1.4 uM); BL = blank control; TS = test sample.

BL	BL	TS	TS
LS1	LS1	...	...
LS2	LS2	...	...
LS3	LS3		
LS4	LS4		
LS5	LS5		
LS6	LS6		
LS7	LS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
LS1-LS7	50 uL	serial dilution (1000 to 1.34 uM)
BL	50 uL	H_2O
TS	50 uL	test sample

1. Prepare Lead Standards (LS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 μL of reagent per well instead of 50 μL.
2. Add 50 uL of Lead Green working solution to each well of Lead Standard, blank control, and test samples to make the total Lead assay volume of 100 uL/well. For a 384-well plate, add 25 uL of working solution into each well instead for a total volume of 50 uL/well.
3. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 490/530 nm.

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EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Pb2+ samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

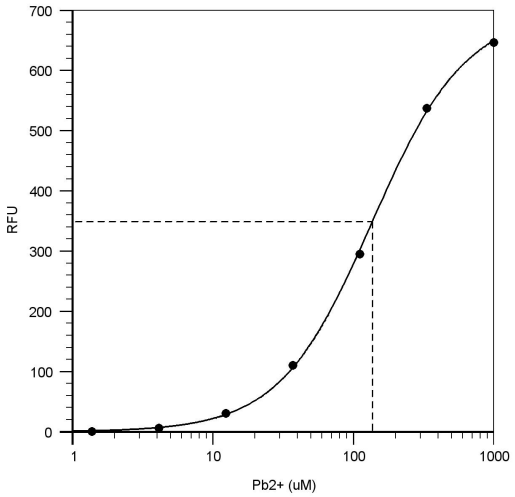

Figure 1. Lead dose response was measured with Amplite™ Fluorimetric Lead (II) Ion Quantitation Kit in a 96-well solid black plate.

DISCLAIMER