Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Green Fluorescence*

Catalog number: 11953
Unit size: 500 Tests

Component	Storage	Amount
Component A: FDP (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component C: Alkaline Phosphatase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder, 10 units)
Component D: DMSO	Freeze (<-15 °C)	1 vial (500 µL)
Component E: Stop Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 mL)

OVERVIEW

Alkaline phosphatase is a highly sensitive enzyme for ELISA, immuno-histochemical, Northern, Southern and Western blot applications. It is widely used in various biological assays (in particular, immunoassays) and ELISA-based diagnostics. This Amplite™ Alkaline Phosphatase Assay Kit uses FDP, a sensitive fluorogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts as well as on solid surfaces (such as PVDF membranes). The kit provides all the essential components with our optimized 'mix and read' assay protocol that is compatible with HTS liquid handling instruments.

AT A GLANCE

Protocol summary

1. Prepare Alkaline Phosphatase working solution (50 µL)
2. Add Alkaline Phosphatase standards or test samples (50 µL)
3. Incubate at RT or 37°C for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm (Cutoff =515 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 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.

1. FDP stock solution (250X):
Add 100 µL of DMSO (Component D) into the vial of FDP (Component A) to make 250X FDF stock solution. The FDP stock solution should be used promptly.

2. Alkaline Phosphatase standard solution (100 U/mL):
Add 100 µL of distilled H_2O with 0.1% BSA (H_2O - 0.1% BSA) into Alkaline Phosphatase Standard (Component C, 10 units) to generate 100 units/mL Alkaline Phosphatase standard solution.

Note The Alkaline Phosphatase standard solution is not stable.

PREPARATION OF STANDARD SOLUTION

Alkaline Phosphatase standard

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/11953

Add 10 µL of 100 units/mL Alkaline Phosphatase standard solution into 990 µL of H_2O - 0.1% BSA to generate a 1,000 mU/mL Alkaline Phosphatase standard solution. Take 1,000 mU/mL Alkaline Phosphatase standard solution and perform 1:10 (AS7) and then 1:3 serial dilutions in H_2O - 0.1% BSA to get serial dilutions of Alkaline Phosphatase standard (AS6 - AS1).

PREPARATION OF WORKING SOLUTION

Add 20 µL of 250X FDP stock solution into 5 mL of Assay Buffer (Component B) and mix well to prepare Alkaline Phosphatase working solution.

Note Keep from light.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Alkaline Phosphatase Standards and test samples in a solid black 96-well microplate. AS = Alkaline Phosphatase Standards (AS1 - AS7, 0.1 to 100 mU/mL); BL=Blank Control; TS=Test Samples.

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilution (0.1 to 100 mU/mL)
BL	50 µL	H_2O - 0.1% BSA
TS	50 µL	test sample

Run Alkaline Phosphatase assay in supernatants:

1. Prepare Alkaline Phosphatase standards (AS), 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.

Note Prepare the cell or tissue samples as desired. Unused serial dilutions of Alkaline Phosphatase standard should be discarded.

2. Add 50 µL of Alkaline Phosphatase working solution to each well of Alkaline Phosphatase standard, blank control, and test samples to make the total Alkaline Phosphatase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Alkaline Phosphatase working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at the desired temperature for 10 to 30 minutes, protected from light. *Optional:* Add 50 µL/well (for a 96-well plate) or 25 µL/well (for a 384-well plate) of Stop Solution (Component E) at the end of 30 minutes incubation.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 490 ± 10, Emission = 525 ± 10 nm (Cutoff =515 nm).

Run Alkaline Phosphatase assay in cells:

1. Treat the cells as desired.

2. Remove the growth medium completely from the cell plate.

Note It is important to remove the growth medium completely from the cell plate due to the interference of the growth medium with the FDP.

3. Make 1:1 dilution of the 5 mL Alkaline Phosphatase working solution with 5 mL distilled H_2O.

4. Add 100 µL (for a 96-well plate) or 50 uL (for a 384-well plate) of 1:1 diluted Alkaline Phosphatase working solution into the cell wells.

5. Incubate the reaction at the desired temperature for 30 to 60 minutes, protected from light. *Optional:* add 50 µL/well (for a 96-well plate) or 25 µL/well (for a 384-well plate) of Stop Solution (Component E) at the end of 30 minutes incubation.

6. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 490 ± 10, Emission = 525 ± 10 nm (Cutoff = 515 nm).

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 ALP samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

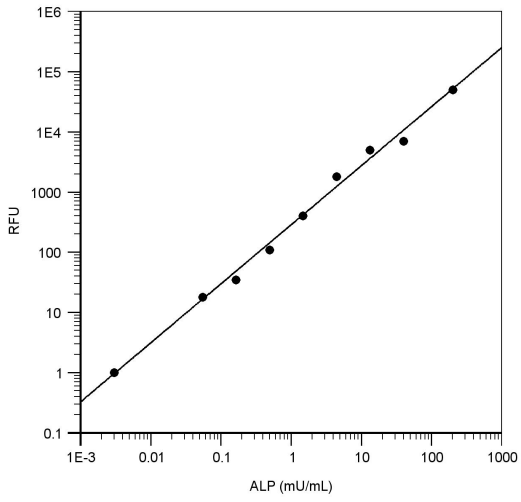

Figure 1. Alkaline phosphatase dose response was measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER