Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Blue Fluorescence*

Catalog number: 11952
Unit size: 500 Tests

Component	Storage	Amount
Component A: MUP Plus™ (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)

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 MUP, a 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 and/or test samples (50 µL)
3. Incubate at RT or 37 °C for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 360/450 nm (Cutoff = 435 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	360 nm
Emission:	450 nm
Cutoff:	435 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. MUP Plus™ stock solution (250X):
Add 100 µL of sterile H_2O into the vial of MUP Plus™ (Component A) to make 250X MUP Plus™ stock solution. The MUP Plus™ stock solution should be used promptly.

2. Alkaline Phosphatase standard solution (100 mU/mL):
Add 100 µL of distilled H2O with 0.1% BSA (H2O-0.1% BSA) to Alkaline Phosphatase Standard (Component C, 10 units) to generate 100 units/mL Alkaline Phosphatase standard solution.

PREPARATION OF STANDARD SOLUTION

Alkaline Phosphatase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11952

Add 10 µL of 100 units/mL Alkaline Phosphatase standard solution into 990 µL of H2O-0.1% BSA to generate 1,000 mU/mL Alkaline Phosphatase standard solution. Take 1,000 mU/mL standard solution to perform 1:10 in H2O-0.1% BSA to get 100 mU/mL standard solution (AS7). Then take 100 mU/mL standard solution (AS7) and perform 1:3 serial dilutions in H2O-0.1% BSA to get serially diluted Alkaline Phosphatase standards (AS6 - AS1).

Note Unused portion of diluted Alkaline Phosphatase standard solution should be discarded.

PREPARATION OF WORKING SOLUTION

Add 20 µL of 250X MUP Plus™ stock solution into 5 mL Assay Buffer (Component B) to make Alkaline Phosphatase working solution.

Note Keep from light. Prepare fresh Alkaline Phosphatase working solution for each experiment.

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 - 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 Dilutions (0.1 to 100 mU/mL)
BL	50 µL	H_2O - 0.1% BSA
TS	50 µL	test sample

Run alkaline phosphate 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.

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.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 360 ± 10 nm, Emission = 450 ± 10 nm (Cutoff = 435 nm).

Run alkaline phosphatase assay in cells:

1. Treat the cells as desired.

2. Add equal volume of Alkaline Phosphatase working solution into each cell well (such as 100 µL/96-well plate, or 50 µL/384-well plate).

   Note Alternatively, remove the growth medium from the cell plate, and make 1:1 dilution of the 5 mL Alkaline Phosphatase working solution with 5 mL distilled H_2O. Then add 100 µL (for a 96-well plate) or 50 uL (for a 384-well plate) of 1:1 diluted working solution into the cell wells.

3. Incubate the reaction at the desired temperature for 30 to 60 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 360 ± 10 nm, Emission = 450 ± 10 nm (Cutoff = 435 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

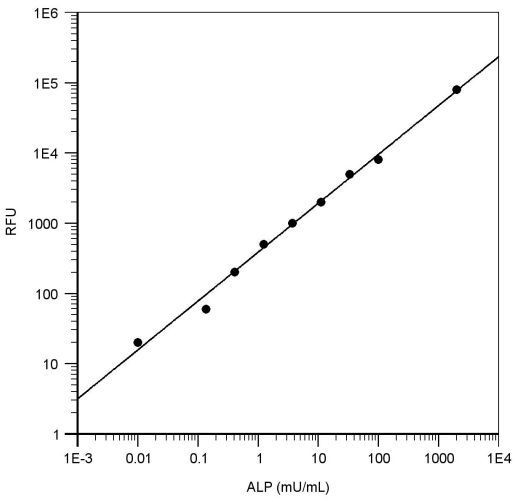

Figure 1. Alkaline phosphatase dose response was measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.01 mU/well of alkaline phosphatase can be detected with 30 minutes incubation (n=3). RFU read at Ex/Em = 360/450 nm (Cutoff = 435 nm).

DISCLAIMER

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