Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Near Infrared Fluorescence*

Catalog number: 11954
Unit size: 500 Tests

Component	Storage	Amount
Component A: SunRed™ Substrate (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 a proprietary flurogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts as well as on solid surfaces (such as PVDF membranes). This proprietary flurogenic phosphatase substrate generates a fluorescent product that has strongly red fluorescence upon interaction with phosphatase. 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 30 to 120 minutes
4. Monitor fluorescence intensity at Ex/Em = 620/660 nm (Cutoff = 630 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	620 nm
Emission:	660 nm
Cutoff:	630 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. SunRed™ Substrate stock solution (250X):
Add 100 µL of double sterile H_2O into the vial of SunRed™ Substrate (Component A) to make 250X SunRed™ Substrate stock solution. The 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 (H2O - 0.1% BSA) to Alkaline Phosphatase Standard (Component C, 10 units) to generate a 100 units/mL Alkaline Phosphatase standard solution.

Note The Alkaline Phosphatase standard solution is not stable.

PREPARATION OF STANDARD SOLUTION

Alkaline Phophatase standard

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/11954

Add 10 µL of 100 units/mL Alkaline Phosphatase standard solution to 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 1:3 serial dilutions to get serially diluted Alkaline Phosphatase standards (AS7 - AS1) with H_2O - 0.1% BSA.

PREPARATION OF WORKING SOLUTION

For one 96-well plate, add 20 µL of 250X SunRed™ Substrate stock solution to 5 mL of Assay Buffer (Component B) and mix well to prepare Alkaline Phosphatase working solution .

Note Keep from light and prepare fresh reaction mixture 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, 0.3 to 300 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 (0.3 to 300 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.

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 for 30 to 120 minutes at the desired temperature, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 620/660 nm (Cutoff = 630 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 SunRed™ Substrate.

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

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

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

6. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 620/660 nm (Cutoff = 630 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 Four Parameter Logistics Calculator which can be found at:

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

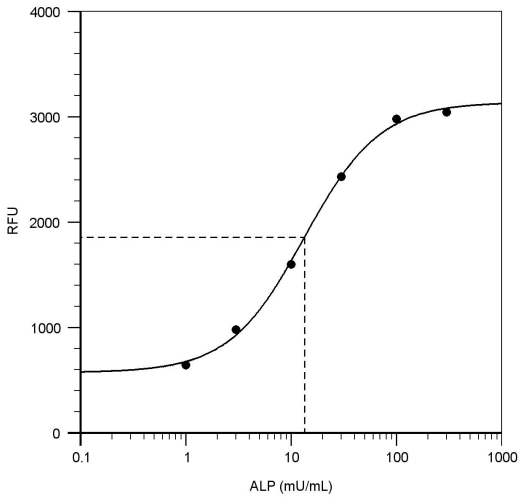

Figure 1. Alkaline phosphatase dose response was measured with the Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER