Amplite™ Luminometric Alkaline Phosphatase Assay Kit *Luminescence*

Catalog number: 11956
Unit size: 100 Tests

Component	Storage	Amount
Component A: Phosphatase Substrate	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component B: Reaction Buffer	Refrigerate (2-8 °C), Minimize light exposure	1 bottle (5 mL)
Component C: Alkaline Phosphatase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder, 10 units)
Component D: Assay Buffer	Freeze (<-15 °C)	1 bottle (5 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 a proprietary luminogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions as well as in cell extracts. This proprietary phosphatase substrate generates a luminescent product that produces strong luminescence 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. It has extremely high sensitivity, and can be used for the assays that require demanding sensitivity.

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 for 30 - 60 minutes
4. Add Assay Buffer (Component D) (50 µL)
5. Incubate at RT for 10 - 30 minutes
6. Monitor luminescence intensity

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

KEY PARAMETERS

Instrument: Luminescence microplate reader
Recommended plate: Solid white

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. Alkaline Phosphatase standard solution (100 U/mL):
Add 100 µL of distilled H_2O with 0.1% BSA (H_2O - 0.1% BSA) into the vial of 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 Phosphatase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11956

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

Note Unused serial dilutions of Alkaline Phosphatase standard should be discarded.

PREPARATION OF WORKING SOLUTION

Mix the whole content of Phosphatase Substrate (Component A) with Reaction Buffer (Component B) to make Alkaline Phosphatase working solution. Protect 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 white 96-well microplate. AS=Alkaline Phosphatase Standards (AS1 - AS7, 0.01 to 10 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 (0.01 to 10 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 for 30 to 60 minutes at room temperature, protected from light.

4. Add 50 µL of Assay Buffer (Component D) into each well of Alkaline Phosphatase standard, blank control, and test samples with assay reaction mixture to make the total Alkaline Phosphatase assay volume of 150 µL/well. For a 384-well plate, add 25 µL of Assay Buffer (Component D) into each well, for a total volume of 75 µL/well.

5. Incubate the reaction for 10 to 30 minutes at room temperature, protected from light.

6. Monitor the luminescence increase with a standard luminescence plate reader.

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 phosphatase 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 the cell wells.

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

6. Add 50 µL (96-well plate) or 25 uL (384-well plate) of Assay Buffer (Component D) into the cell wells containing working solution.

7. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

8. Monitor the luminescence increase with a standard luminescence plate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RLU) 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

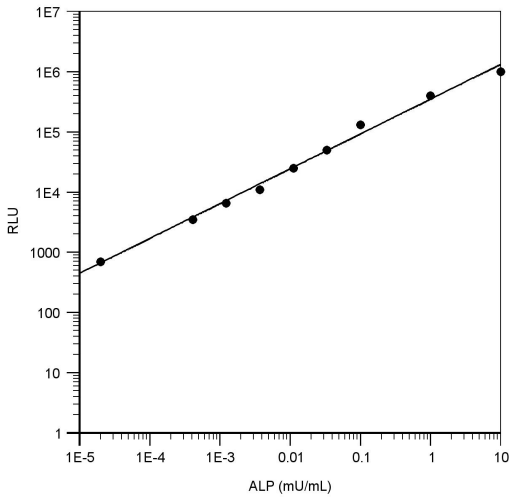

Figure 1. Alkaline phosphatase dose response was measured with Amplite™ Luminometric Alkaline Phosphatase Assay Kit in a white 96-well plate using a NovoStar microplate reader (BMG Labtech). As low as 0.001 mU/mL alkaline phosphatase can be detected with 20 minutes incubation (n=3).

DISCLAIMER