Amplite™ Luminometric Alkaline Phosphatase Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11956 (100 assays)	Keep in freezer Avoid exposure to light	Luminescence microplate readers

Introduction

Alkaline phosphatase (ALP) (EC 3.1.3.1) 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.

Amplite™ Luminometric Alkaline Phosphatase Assay Kit uses D-luciferin phosphate as the luminogenic phosphatase substrate to quantify alkaline phosphatase activity in solutions and in cells. D-luciferin phosphate is not recognized by luciferase until its phosphate group is removed to give luciferin. The kit provides all the essential components with an optimized "mix and read" assay protocol which is compatible with HTS liquid-handling instruments. Our Amplite™ Luminometric Alkaline Phosphatase Assay Kit can be readily performed in a 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read using luminescence microplate readers. The high sensitivity makes the kit ideal for the assays that require low detection limit.

Kit Key Features

Optimized:	Optimized conditions for detecting alkaline phosphatase activity.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Phosphatase Substrate	1 vial (lyophilized powder)
Component B: Reaction Buffer	1 bottle (5 mL)
Component C: Alkaline Phosphatase Standard	1 vial (lyophilized powder, 10 units)
Component D: Assay Buffer	1 bottle (5 mL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare assay reaction mixture (50 µL) → Add alkaline phosphatase standards and/or test samples (50 µL) → Incubate at RT for 30 - 60 minutes → Add assay buffer (50 µL) → Incubate at RT for 10 - 30 minutes → Monitor luminescence intensity

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

1. Prepare assay reaction mixture:

Mix the whole content of Phosphatase Substrate (Component A) with Reaction Buffer (Component B) and keep from light.

2. Prepare serially diluted alkaline phosphatase standards (0 to 100 mU/mL):

2.1 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. Unused solution should be aliquoted and stored at -20 °C. Avoid repeated freeze and thaw cycles.*

2.2 Add 10 µL of 100 units/mL alkaline phosphatase standard solution (from Step 2.1) into 990 µL of H_2O-0.1% BSA to generate a 1,000 mU/mL alkaline phosphatase standard solution.

2.3 Take 100 µL of 1,000 mU/mL alkaline phosphatase standard solution (from Step 2.2) to perform 1:100 and then 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0 mU/mL serially diluted alkaline phosphatase standards.

2.4 Add serially diluted alkaline phosphatase standards and/or alkaline phosphatase containing test samples into a solid white 96-well microplate as described in Tables 1 and 2.
Note 1: Prepare cells or tissue samples as desired.
Note 2: Unused serial dilutions of alkaline phosphatase standard should be discarded.

Table 1. Layout of alkaline phosphatase standards and test samples in a solid white 96-well microplate

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

Note: AS = Alkaline Phosphatase Standards; BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

Alkaline Phosphatase Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	H_2O-0.1% BSA: 50 µL	50 µL

**Note: Add serially diluted alkaline phosphatase standards from 10 to 0.01 mU/mL into wells from AS1 to AS7 in duplicate.*

3. Run alkaline phosphatase assay in supernatants:

3.1 Add 50 µL of assay reaction mixture (from Step 1) into each well of alkaline phosphatase standard, blank control, and test samples (see Step 2.4, Table 1) to make the total alkaline phosphatase assay volume of 100 µL/well
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

3.2 Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.

3.3 Add 50 µL of Assay Buffer (Component D) into each well of alkaline phosphatase standard, blank control, and test samples with assay reaction mixture (see Step 3.2) to make the total alkaline phosphatase assay volume of 150 µL/well
Note: For a 384-well plate, add 25 µL of Assay Buffer (Component D) into each well.

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

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

4. Run alkaline phosphatase assay in cells:

4.1 Treat the cells as desired.

4.2 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.

4.3 Make 1:1 dilution of the 5 mL assay reaction mixture (from Step 1) with 5 mL distilled H_2O.

4.4 Add 100 µL (96-well plate) or 50 uL(384-well plate) of 1:1 diluted assay reaction mixture (from Step 4.3) into the cell wells (from Step 4.2).

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

4.6 Add 50 µL (96-well plate) or 25 uL(384-well plate) of Assay Buffer (Component D) into the cell wells containing assay reaction mixture (from Step 4.5).

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

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

Data Analysis

The luminescence in blank wells (with the reaction buffer only) is used as a control, and is subtracted from the values for those wells with alkaline phosphatase reactions. An alkaline phosphatase standard curve is shown in Figure 1. *Note: The luminescence background increases with time due to spontaneous hydrolysis, thus it is important to subtract the luminescence intensity value of the blank wells for each data point.*

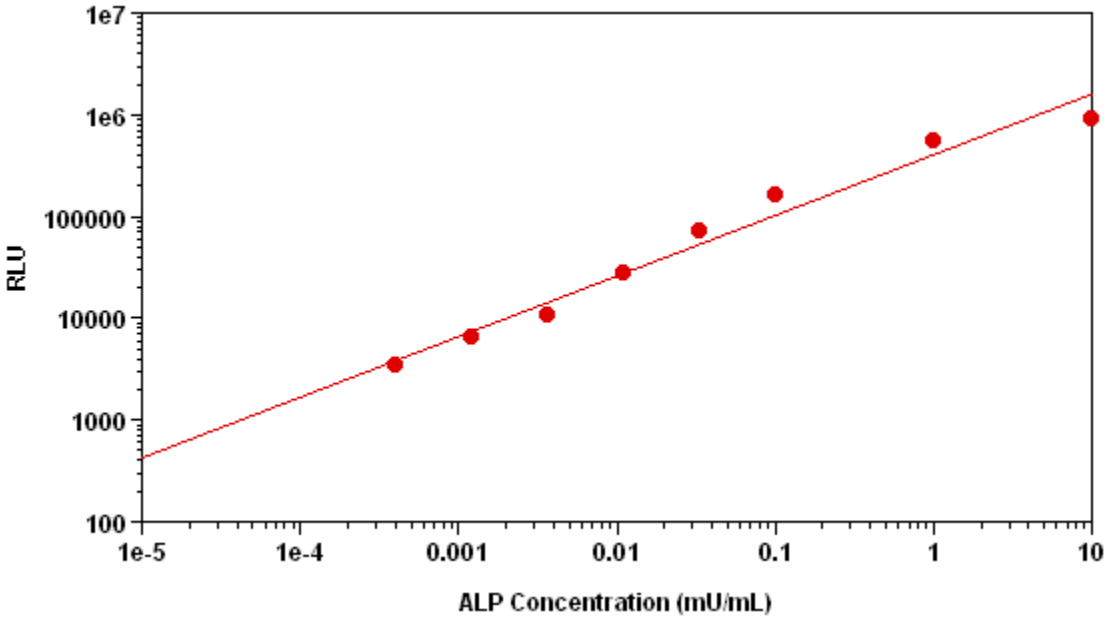

Figure 1. Alkaline phosphatase dose response was measured with the Amplite™ Luminometric Alkaline Phosphatase Assay Kit in a white 96-well plate using a NovoStar microplate reader (BMG Labtech). As low as 0.001 mU/mL alkaline phosphatase can be detected with 20 minutes incubation (n=3).

References

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