Amplite™ Luminometric Alkaline Phosphatase Assay Kit

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #11956 (100 assays)	Keep in freezer and avoid light	Luminescence microplate readers

Introduction

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 our propreitary luminogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cells, 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.

This Amplite™ Alkaline Phosphatase Assay Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by luminescence microplate readers. The characteristic of extremely high sensitivity of the kit can be used for the assays that require demanding sensitivity.

Kit Key Features

Optimized:	Optimized conditions for detecting alkaline phosphatase activity.
Continuous:	Easily adapted to automation with no separation required.
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 or test samples (50 µL) → Incubate at RT for 30-60 min→Add assay buffer (50 µL) → Incubate at RT for 10-30 min → Read luminescence intensity

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

1. Prepare assay reaction mixture:

1.1 Mixing the whole content of Phosphatase Substrate (Component A) with Reaction Buffer (Component B). Kept from light.

2. Prepare serial alkaline phosphatase (0 to 100 mU/mL) solutions:

2.1 Add 100 µL of distilled H_2O with 0.1% BSA (H_2O-0.1% BSA) to alkaline phosphatase standard (Component C, 10 units) to generate 100 units/mL standard solution.
Note: The alkaline phosphatase stock solution is not stable, aliquoted and stored unused stock solution at -20°C, avoid repeated freeze-thaw cycles.

2.2 Take10 µL of 100 units/mL alkaline phosphatase standard solution (from Step 2.1) to 990 µl of H_2O-0.1% BSA to generate 1,000 mU/mL solution.

2.3 Take 100 µL of 1,000 mU/mL 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 standard alkaline phosphatase solutions.

2.4 Add alkaline phosphatase standards and alkaline phosphatase containing test samples into a solid white 96-well microplate as described in Tables 2 and 3.
Note 1: Prepare your cell or tissue samples as desired.
Note 2: Unused portion of diluted alkaline phosphatase solution 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 Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	H_2O-0.1% BSA: 50 µL	50 µL

**Note: Add the serially diluted alkaline phosphatase standards from 10 mU 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 1.1) to each well of the alkaline phosphatase standard, blank control, and test samples (see Step 2.3, Table 2) so that the total alkaline phosphatase assay volume is 100 µL/well
Note: For a 384-well plate, add 25 µL sample, and 25 µL of assay reaction mixture per 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) to each well of the alkaline phosphatase standard, blank control, and test samples with assay reaction mixture (see Step 3.2) so that the total alkaline phosphatase assay volume is 150 µL/well
Note: For a 384-well plate, add 25 µL Assay Buffer (Component D) per well.

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

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

4. Run alkaline phosphatase assay in cells:

4.1 Treat your 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 1.1) with 5 mL distilled H_2O.

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

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

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

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

4.8 Monitor the luminescence increase using 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. The typical data are shown in Figure 1 (alkaline phosphatase standard curve).

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.

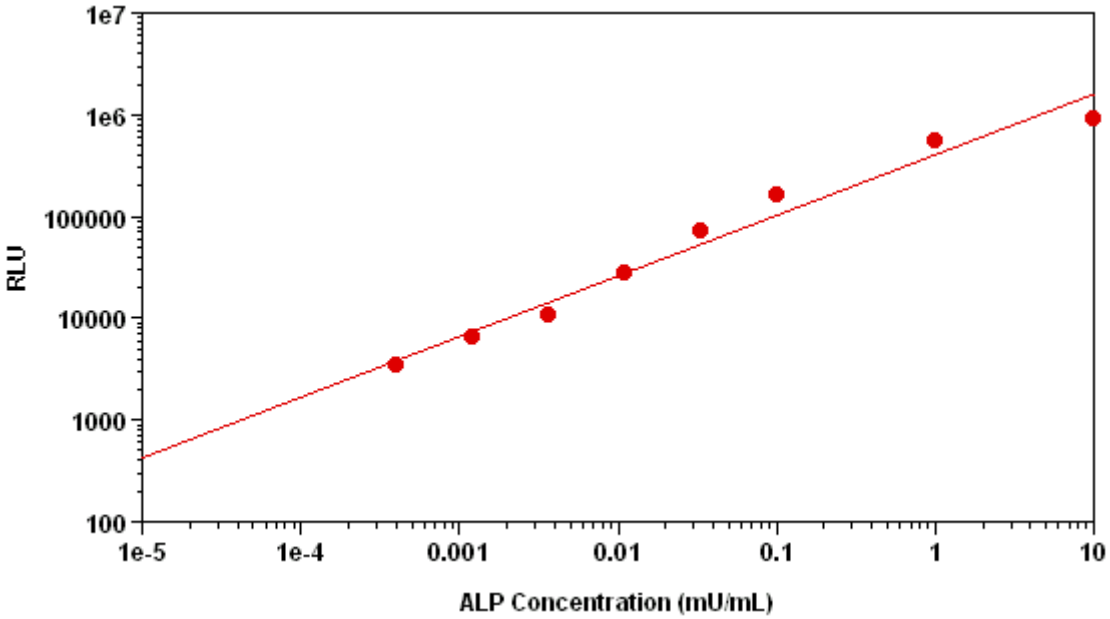

Figure 1. Alkaline phosphatase dose response on 96-well white plate using a NovoStar microplate reader (BMG Labtech) measured with the Amplite™ Alkaline Phosphatase Assay Kit. As low as 0.001 mU/well of alkaline phosphatase can be detected with 20 minutes incubation time (n=3).

References:

1. Zhu X, Jiang C. (2006) 8-Quinolyl phosphate as a substrate for the fluorimetric determination of alkaline phosphatase. Clin Chim Acta.
2. Ali AT, Penny CB, Paiker JE, Psaras G, Ikram F, Crowther NJ. (2006) The effect of alkaline phosphatase inhibitors on intracellular lipid accumulation in preadipocytes isolated from human mammary tissue. Ann Clin Biochem, 43, 207.
3. Lee DH, Lim BS, Lee YK, Yang HC. (2006) Effects of hydrogen peroxide (H_2O_2) on alkaline phosphatase activity and matrix mineralization of odontoblast and osteoblast cell lines. Cell Biol Toxicol, 22, 39.
4. Ali AT, Penny CB, Paiker JE, van Niekerk C, Smit A, Ferris WF, Crowther NJ. (2005) Alkaline phosphatase is involved in the control of adipogenesis in the murine preadipocyte cell line, 3T3-L1. Clin Chim Acta, 354, 101.
5. Rieu JP, Ronzon F, Place C, Dekkiche F, Cross B, Roux B. (2004) Insertion of GPIanchored alkaline phosphatase into supported membranes: a combined AFM and fluorescence microscopy study. Acta Biochim Pol, 51, 189.