Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit

Red Fluorescence

Ordering Information:
Product Number: #11954 (500 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in freezer and avoid light

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 Phospholite™ Red, a fluorogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts, in live 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 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 either fluorescence microplate reader with Ex/Em = 530 to 570 nm/ 590 to 600 nM (maximum Ex/Em = 570 nm/590 nm)

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

Component	Amount
Component A: Phospholite™ Red (light sensitive)	1 vial
Component B: Assay buffer	1 bottle (50 mL)
Component C: Alkaline phosphatase standard	1 vial (50 µL, 10 µg/mL)
Component D: DMSO	1 vial (500 µL)

Assay Protocol (for 1 plate)

Brief Summary

Prepare assay reaction mixture (50 µL) → Add alkaline phosphatase standards or test samples (50 µL) → Incubate at RT or 37°C for 10-30 min → Read fluorescence at Ex 570 nm/Em 590 nm

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

1. Prepare Phospholite™ Red stock solutions:

1.1 Phospholite™ Red stock solution (250X): Add 100 µL of DMSO (Component D) into the vial of Phospholite™ Red (Component A). The stock solution should be used promptly. Any remaining solution need be aliquoted and refrozen at -20°C.
Note: Avoid repeated freeze-thaw cycles.

2. Prepare assay reaction mixture:

2.1 Prepare Assay reaction mixture according to the following table and kept from light:

Table 1. Assay reaction mixture for one 96-well plate (2X)

Components	**Volume**
Phospholite™ Red (250X, from step 1.1)	20 uL
Assay buffer (Component B)	5 mL
Total volume	5 mL

3. Prepare serial alkaline phosphatase (0 to 10 ng/mL) solutions

3.1 Add 5 µL of alkaline phosphatase standard (Component C, 10 µg/mL) to 995 µl Assay buffer (Component B) or buffer of your choice (avoid phosphate buffer if possible) to generate 50 ng/mL standard.

3.2 Take 200 µL of 50 ng/mL solution to perform 1:5 and then 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.0001 and 0 standard alkaline phosphatase solutions.

3.3 Add alkaline phosphatase standards and alkaline phosphatase -containing test samples into a 96-well solid black 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 2. Layout of Alkaline Phosphatase Standards and test samples in a solid black 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 3. Reagent composition for each well:

Alkaline Phosphatase Standard	Blank Control	Test Sample
Serial dilutions* (50 µL)	Assay buffer (Component B): 50 µL	50 µL

**Note1: Add the serially diluted alkaline phosphatase standards from 3 ng to 0.003ng/mL into wells from AS1 to AS7 in duplicate.*

4. Run alkaline phosphatase assay in supernatants

4.1 Add 50 µL of assay reaction mixture (from step 2) to each well of the alkaline phosphatase standard, blank control, and test samples (see step 3, table 3) so that the total alkaline phosphatase assay volume is 100 µL/well

Note: For a 384-well plate, add 25 µL sample, 25 µL of assay reaction mixture *per well.*

4.2 Incubate the reaction for 10 to 30 minutes at the desired temperature, protected from light.

4.3 Monitor the fluorescence increase with 570±30 nm excitation and 590±20 nm emission using a fluorescence plate reader.

5. Run Data Analysis

The fluorescence in blank wells (with the assay 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 fluorescence background increases with time due to spontaneous hydrolysis, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

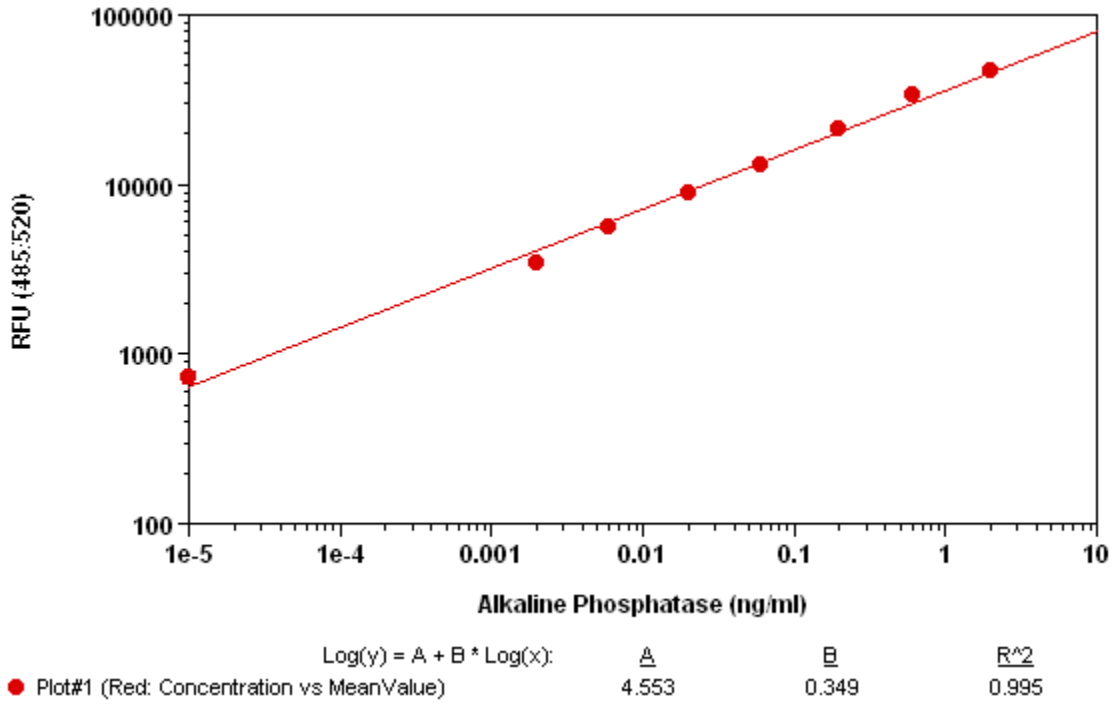

Figure 1. Alkaline phosphatase dose response on 96-well black plate using a NovoStar microplate reader (BMG Labtech) measured with the Amplite™ Alkaline Phosphatase Assay Kit. As low as 0.2 pg of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

References:

1. Zhu X, Jiang C. (2006) 8-Quinolyl phosphate as a substrate for the fluorimetric determination of alkaline phosphatase. Clin Chim Acta.
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3. Lee DH, Lim BS, Lee YK, Yang HC. (2006) Effects of hydrogen peroxide (H(2)O(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.
6. Palermo C, Manduca P, Gazzerro E, Foppiani L, Segat D, Barreca A. (2004) Potentiating role of IGFBP-2 on IGF-II-stimulated alkaline phosphatase activity in differentiating osteoblasts. Am J Physiol Endocrinol Metab, 286, E648.

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