

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit

Near Infrared Fluorescence

Ordering Information:

Product Number: #11954 (500 assays)

Storage Conditions:

Keep in freezer and avoid light

Instrument Platform:

Fluorescence 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 Quest Red™ phosphate, a fluorogenic 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 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 fluorescence microplate reader with Ex/Em = 630±10/660±10 nm (maximum Ex/Em = 620/660 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

Components	Amount
Component A: Quest Red™ Phosphate (light sensitive)	1 vial
Component B: Assay Buffer	1 bottle (25 mL)
Component C: Alkaline Phosphatase Standard	1 vial (lyophilized powder, 10 units)

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 or 37°C for 30 to 120 min → Read fluorescence at Ex/Em = 620/660 nm

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

1. Prepare Quest Red™ Phosphate stock solutions:

- 1.1 **Quest Red™ Phosphate stock solution (250X):** Add 100 µL of double sterile H₂O into the vial of Quest Red™ Phosphate (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
Quest Red™ phosphate (250X, from Step 1.1)	20 µL
Assay buffer (Component B)	5 mL
Total volume	5 mL

Note: Prepare fresh reaction mixture for each experiment.

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

- 3.1 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.
- 3.2 Take 10 μL of 100 units/mL alkaline phosphatase standard solution (from Step 3.1) to 990 μL of H_2O -0.1% BSA to generate 1,000 mU/mL solution.
- 3.3 Take 100 μL of 1,000 mU/mL solution (from Step 3.2) to perform 1:10 and then 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3, 0.1, and 0 mU/mL standard alkaline phosphatase solutions.
- 3.4 Add alkaline phosphatase standards and alkaline phosphatase containing test samples into a solid black 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 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)	H_2O -0.1% BSA: 50 μL	50 μL

Note: Add the serially diluted alkaline phosphatase standards from 100 mU to 0.01 mU/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 3.3, Table 3) so that the total alkaline phosphatase assay volume is 100 μL /well.
Note: For a 384-well plate, add 25 μL sample, and 25 μL of assay reaction mixture per well.
- 4.2 Incubate the reaction for 30 to 120 minutes at the desired temperature, protected from light.
- 4.3 Monitor the fluorescence increase with $\text{Ex/Em} = 630 \pm 10 / 660 \pm 10$ nm using a fluorescence plate reader.

5. Run alkaline phosphatase assay in cells:

- 5.1 Treat your cells as desired.
- 5.2 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 Quest Red™ Phosphate.
- 5.3 Make 1:1 dilution of the 5 mL assay reaction mixture (from Step 2.1, Table 2) with 5 mL distilled H_2O .
- 5.4 Add 100 μL (for 96-well plate) or 50 μL (for 384-well plate) of 1:1 diluted assay reaction mixture (from Step 5.3) to the cell wells (from Step 5.2).
- 5.5 Incubate the reaction for 30 to 60 minutes at the desired temperature, protected from light.
- 5.6 Monitor the fluorescence increase with $\text{Ex/Em} = 630 \pm 10 / 660 \pm 10$ nm using a fluorescence plate reader.

Data Analysis

The fluorescence in blank wells (with equal volume of assay reaction mixture and H₂O-0.1%BSA 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.

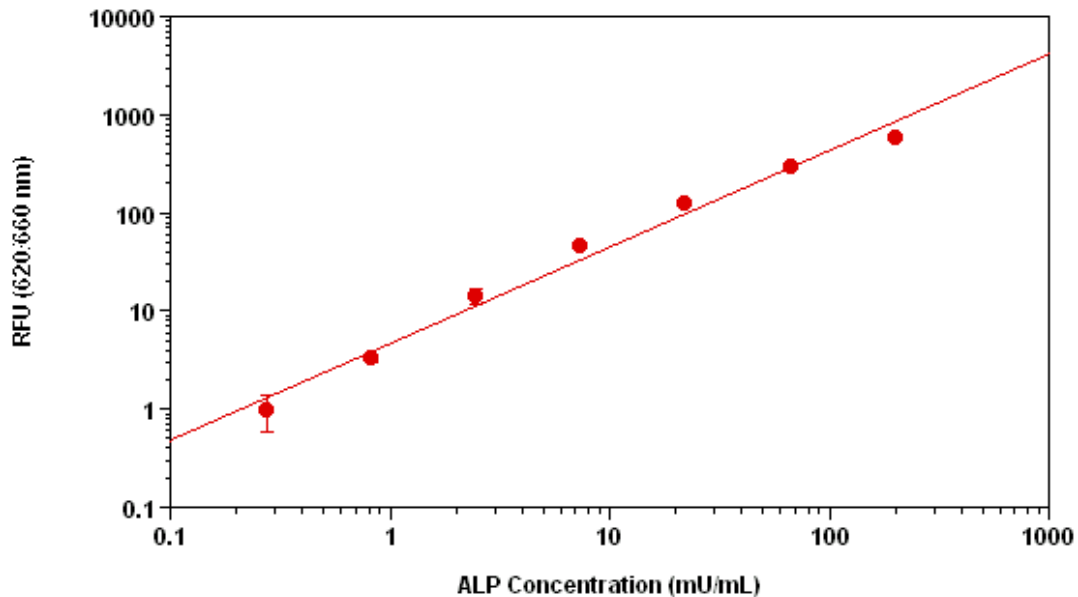


Figure 1. Alkaline phosphatase dose response on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices) measured with the Amplite™ Alkaline Phosphatase Assay Kit with Ex/Em = 620/660 nm (cut off = 630 nm). As low as 0.3 mU/well of alkaline phosphatase can be detected with 60 minutes incubation time (n=3).

References:

1. Zhu X, Jiang C. (2006) 8-Quinoyl 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 (H₂O₂) 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.

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