Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11952 (500 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

This Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit uses our MUP Plus™-based coumarin substrate. Similar to MUP, MUP Plus™ is sensitive to phosphatase-induced hydrolysis, giving the halogenated coumarin that possesses intense blue fluorescence. Its almost identical spectral properties to those of MUP enables MUP Plus™ substrates readily compatible with many fluorescence instrument systems equipped with MUP settings. Compared to MUP, MUP Plus™ gives the coumarin fluorophore that has substantially lower pKa, making the MUP Plus™ assay much less pH-dependent.

Our Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit uses our MUP Plus™, a fluorogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts, and on solid surfaces (such as PVDF membranes). It can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = ~360/450 nm. The kit provides an optimized "mix and read" assay protocol which is compatible with HTS liquid handling instruments.

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: MUP Plus™ (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 and/or test samples (50 µL) → Incubate at RT or 37 °C for 10 - 30 minutes → Monitor fluorescence intensity at Ex/Em = 360/450 nm

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

1. Prepare 250X MUP Plus™ stock solutions:

Add 100 µL of sterile H_2O into the vial of MUP PLus™ (Component A). The MUP PLus™ stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at - 20 °C.
Note: Avoid repeated freeze-thaw cycles.

2. Prepare assay reaction mixture:

Prepare assay reaction mixture according to the following table and keep from light.

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Table 1 Assay reaction mixture for one 96-well plate

Components	**Volume**
MUP Plus™ (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.

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

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 a 100 units/mL alkaline phosphatase standard solution.
Note: The alkaline phosphatase standard solution is not stable. Aliquot and store unused solution at - 20 °C. Avoid repeated freeze-thaw cycles.

3.2 Add 10 µL of 100 units/mL alkaline phosphatase standard solution (from Step 3.1) into 990 µL of H_2O-0.1% BSA to generate a 1,000 mU/mL alkaline phosphatase standard 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 serial dilutions of alkaline phosphatase standard.

3.4 Add serially diluted alkaline phosphatase standards and/or alkaline phosphatase containing test samples into a solid black 96-well microplate as described in Tables 2 and 3.
Note 1: Prepare cells or tissue samples as desired.
Note 2: Unused portion of diluted alkaline phosphatase standard 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 Standards	**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 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) into each well of alkaline phosphatase standard, blank control, and test samples (see Step 3.4, Table 3) to get 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.

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

4.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 360 ± 10/450 ± 10 nm.

5. Run alkaline phosphatase assay in cells:

5.1 Treat the cells as desired.

5.2 Add equal volume of assay reaction mixture (from Step 2) into each cell well (such as 100 µL/96-well plate, or 50 µL/384-well plate).

Note: Alternatively, remove the growth medium from the cell plate, and make 1:1 dilution of the 5 mL assay reaction mixture (from Step 2, Table 1) with 5 mL distilled H_2O. Then Add 100 µL (for a 96-well plate) or 50 uL (for a 384-well plate) of 1:1 diluted assay reaction mixture into the cell wells (from Step 5.2).

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

5.4 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 360±10/450±10 nm.

Data Analysis

The fluorescence in blank wells (with equal volume of assay reaction mixture and H_2O-0.1%BSA 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 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.

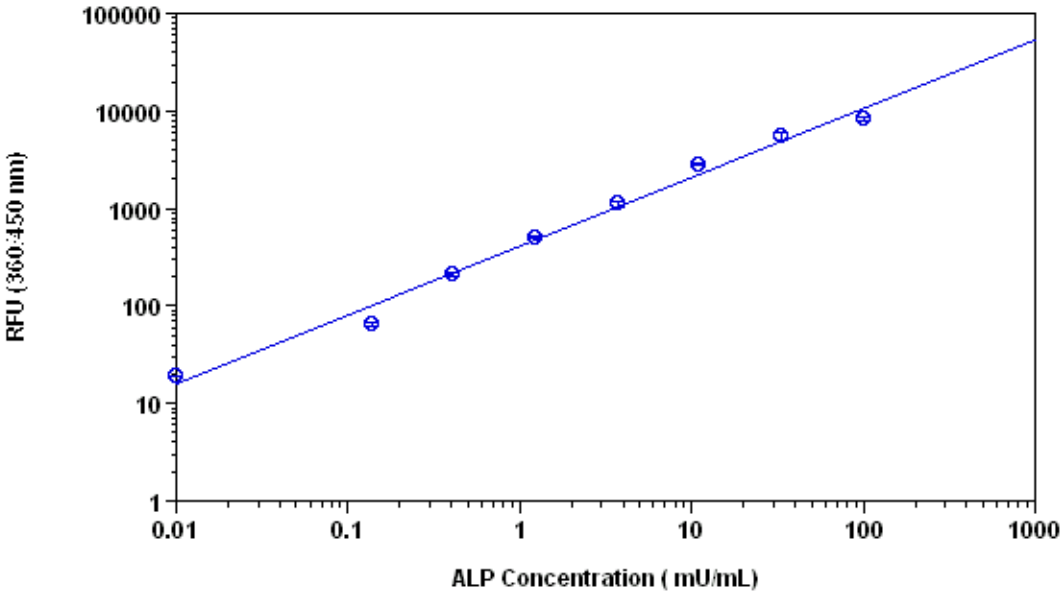

Figure 1 Alkaline phosphatase dose response was measured with the Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.01 mU/well of alkaline phosphatase can be detected with 30 minutes incubation (n=3).

References

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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.