# Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit
**Green Fluorescence**

| *Ordering Information* | *Storage Conditions* | *Instrument Platform* |
|---|---|---|
| Product Number: 11953 (500 assays) | Keep in freezer<br>Avoid exposure to light | Fluorescence microplate readers |

## Introduction

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. An important use of alkaline phosphatase is as a label for enzyme immunoassays. Alkaline phosphatase is a highly sensitive enzyme for ELISA, immuno-histochemical as well as Northern, Southern and Western blot applications. It is widely used in various biological assays (in particular, immunoassays) and ELISA-based diagnostics.

Our Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit uses our FDP, a fluorogenic phosphatase substrate, to quantify the 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 and easily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = ~490/525 nm. The kit provides an optimized "mix and read" assay protocol which is compatible with HTS liquid handling instruments.

## Kit Components

| Components | Amount |
|---|---|
| Component A: FDP (light sensitive) | 1 vial |
| Component B: Assay Buffer | 1 bottle (25 mL) |
| Component C: Alkaline Phosphatase Standard | 1 vial (lyophilized powder, 10 units) |
| Component D: DMSO | 1 vial (500 µL) |
| Component E: Stop Solution | 1 bottle (25 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 or 37 °C for 10-30 minutes → Monitor fluorescence intensity at Ex/Em = 490/ 525 nm**

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

**1. Prepare 250X FDP stock solution:**

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

**2. Prepare assay reaction mixture:**

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

**Table 1**. Assay reaction mixture for one 96-well plate

| Components | Volume |
|---|---|
| 250X FDP (from Step 1) | 20 µL |
| Assay Buffer (Component B) | 5 mL |
| Total volume | 5 mL |

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**3. Prepare serial dilutions of alkaline phosphatase standard (0 to 100 mU/mL):**

3.1 Add 100 µL of distilled $H_2O$ with 0.1% BSA ($H_2O$-0.1% BSA) into 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 standard solution should be aliquoted and stored at -20 $^oC$. Avoid repeated freeze and 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 alkaline phosphatase standard 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 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 the cell or tissue samples as desired.*
*Note 2: Unused serial dilutions of alkaline phosphatase standard 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 serial dilutions of alkaline phosphatase standard 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) into each well of alkaline phosphatase standard, blank control, and test samples (see Step 3.3, Table 3) 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.*

4.2 Incubate the reaction at the desired temperature for 10 to 30 minutes, protected from light.
*Optional: add 50 µL/well (for a 96-well plate) or 25 µL/well (for a 384-well plate) of stop solution (Component E) at the end of 30 minutes incubation.*

4.3 Monitor the fluorescence increase at Ex/Em = 490±10/525±10 nm with a fluorescence plate reader.

**5. Run alkaline phosphatase assay in cells:**

5.1 Treat the 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 FDP.*

5.3 Make 1:1 dilution of the 5 mL assay reaction mixture (from Step 2, Table 1) with 5 mL distilled $H_2O$.

5.4 Add 100 µL (for a 96-well plate) or 50 uL(for a 384-well plate) of 1:1 diluted assay reaction mixture (from Step 5.3) into the cell wells (from Step 5.2).

5.5 Incubate the reaction at the desired temperature for 30 to 60 minutes, protected from light.
*Optional: add 50 µL/well (for a 96-well plate) or 25 µL/well (for a 384-well plate) of stop solution (Component E) at the end of 30 minutes incubation.*

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5.6 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 490 ± 10/525 ± 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.*

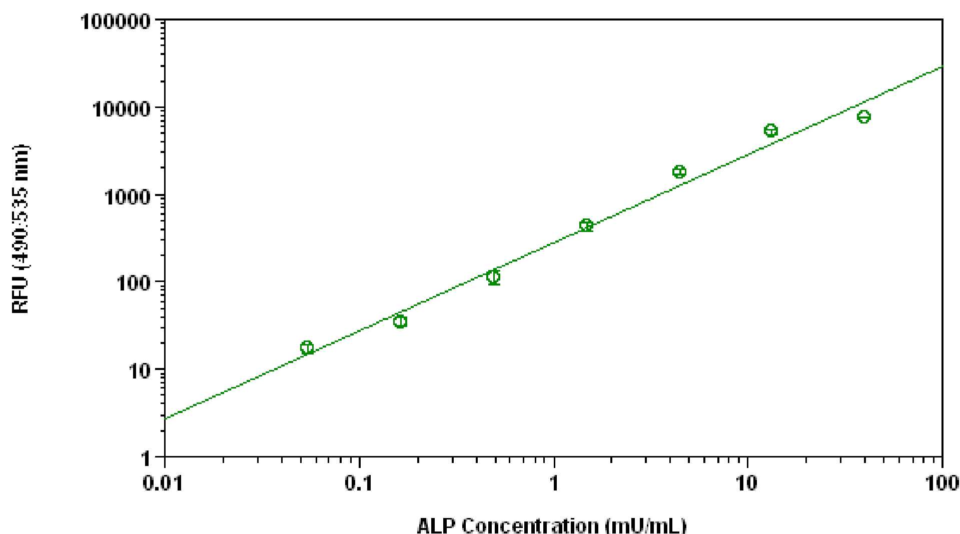


**Figure 1.** Alkaline phosphatase dose response was measured with the Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.01 mU/well of alkaline phosphatase can be detected with 30 minutes incubation (n=3).

## References

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