Amplite™ Colorimetric Alkaline Phosphatase Assay Kit *Yellow Color*

Catalog number: 11950
Unit size: 500 Tests

Component	Storage	Amount
Component A: pNPP (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component C: Alkaline Phosphatase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder, 10 units)

OVERVIEW

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 pNPP, a chromogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts 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.

AT A GLANCE

Protocol summary

1. Prepare alkaline phosphatase standards and/or test samples (50 µL)
2. Add ALP working solution (50 µL)
3. Incubate at RT or 37°C for 10 - 30 minutes
4. Monitor absorbance increase at 400 nm

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	400 nm
Recommended plate:	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. *p*NPP stock solution (100X):
Add 300 µL of distilled H_2O into the vial of *p*NPP (Component A). Mix well. The pNPP stock solution should be used promptly. *Note:* The solution should be good for 3 - 4 weeks if stored properly.

2. Alkaline Phosphate standard solution:
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.

PREPARATION OF STANDARD SOLUTION

Alkaline Phosphate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11950

Add 10 µL of 100 units/mL Alkaline Phosphatase standard solution to 990 µL of H_2O - 0.1% BSA to generate a 1,000 mU/mL Alkaline Phosphatase standard solution. Then take 100 µL of 1,000 mU/mL Alkaline Phosphatase standard solution to perform a 1:10 dilution to obtain 100 mU/mL Alkaline Phosphatase standard solution (AS7). Then perform 1:3 serial dilution to obtain remaining standards (AS6 - AS1). Note: The unused portion of diluted alkaline phosphatase standard solution should be discarded.

PREPARATION OF WORKING SOLUTION

Add 50 µL of pNPP Stock solution (100X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.05 mL of *p*NPP working solution.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Alkaline phosphatase standards and samples in a white/clear bottom 96-well microplate. AS = Alkaline Phosphatase Standards (AS1 - AS7, 0.1 to 100 mU/mL); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilution (0.1 to 100 mU/mL)
BL	50 µL	H_2O - 0.1% BSA
TS	50 µL	test sample

In supernatants:

1. Prepare alkaline phosphate standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL *p*NPP working solution to each well of alkaline phosphate standard, blank control, and test samples to make the total alkaline phosphate assay volume of 100 µL/well. For a 384-well plate, add 25 µL of pNPP working solution into each well instead, for a total volume of 50 µL/well.

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

4. Monitor the absorbance increase with an absorbance plate reader at 400 nm.

In cells:

1. Treat the cells as desired.

2. Add equal volume of *p*NPP working solution 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 working solution 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 working solution to the cell wells.

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

4. Monitor the absorbance increase with an absorbance plate reader at 400 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ALP samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

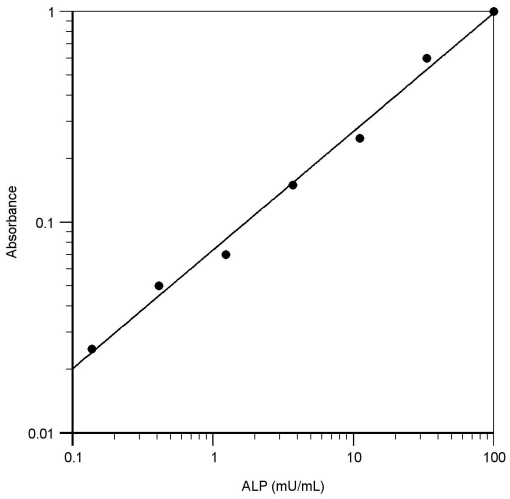

Figure 1. Alkaline phosphatase dose response was measured with the Amplite™ Colorimetric Alkaline Phosphatase Assay Kit in a white/clear bottom 96-well plate using a NovoStar microplate reader (BMG Labtech).

DISCLAIMER