Amplite™ Colorimetric Alkaline Phosphatase Assay Kit

Yellow Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11950 (500 assays)	Keep in freezer Avoid exposure to light	Absorbance 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. The process of removing the phosphate group is called dephosphorylation. 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™ Colorimetric Alkaline Phosphatase Assay Kit uses pNPP, a chromogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts, and on solid surfaces (such as PVDF membranes). The kit provides an optimized "mix and read" assay protocol which is compatible with HTS liquid handling instruments. Its signal can be easily read by an absorbance microplate reader at around 400 nm. This Amplite™ Colorimetric Alkaline Phosphatase Assay Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

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: *p*NPP (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 5 - 30 minutes → Monitor absorbance increase at 400 nm

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

1. Prepare 100X *p*NPP stock solutions:

Add 300 µL of distilled H_2O into the vial of *p*NPP (Component A). Mix the reagents well. The *p*NPP stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 °C.
Note: Avoid repeated freeze-thaw cycles. The solution should be good for 3 - 4 weeks if stored at -20 °C.

2. Prepare *p*NPP reaction mixture:

Prepare *p*NPP reaction mixture according to the following table and keep from light.

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Table 1. *p*NPP Reaction Mixture for one 96-well plate

Components	**Volume**
*p*NPP stock solution (100X, from Step 1)	50 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. The unused solution should be aliquoted and stored 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) to 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 serially diluted Alkaline Phosphatase Standards.

3.4 Add serially diluted alkaline phosphatase standards and alkaline phosphatase containing test samples into a white/clear bottom 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 samples in a white/clear bottom 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 100 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 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.

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

Data Analysis

The absorbance in blank wells (with equal volume of *p*NPP 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.

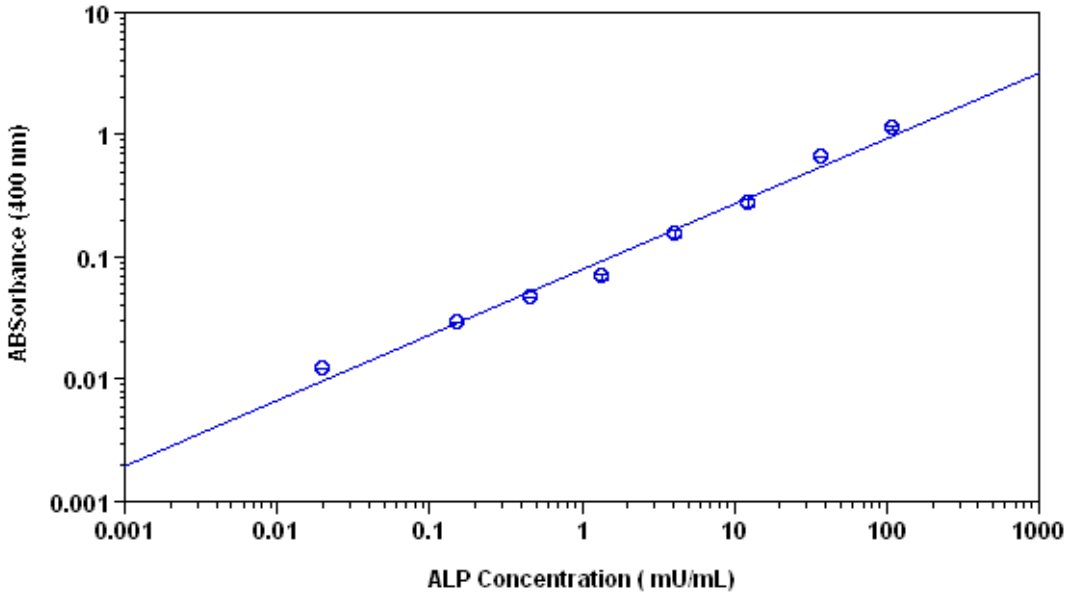

Figure 1. Alkaline phosphatase dose response was measured with the Amplite™ Colorimetric Alkaline Phosphatase Assay Kit in a white/clear bottom 96-well plate using a NovoStar microplate reader (BMG Labtech). As low as 0.03 mU/well of alkaline phosphatase can be detected with 30 minutes incubation (n=3).

References

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