Colorimetric Alkaline Phosphatase Assay Kit, Yellow Color

Product Description

Name :	**Colorimetric Alkaline Phosphatase Assay Kit, *Yellow Color***	
Catalog Number :	FP-JQ6720 500 assays	
Components :	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)

Storage: Keep in freezer. Protect from light and moisture

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 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.

This Alkaline Phosphatase Assay Kit 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 absorbance microplate reader around 400 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.

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Directions for use

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 5-30 min → Read absorbance at 400 nm

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

Note: 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. Prepare *p*NPP stock solutions:

1.1 *p*NPP stock solution (100X): Add 300 µL of distilled H_2O into the vial of *p*NPP (Component A). Mix the reagents well. The stock solution should be used promptly. Any remaining solution need be aliquoted and refrozen at -20°C.

Note: It will be good for 3-4 weeks if stored at -20°C.

2. Prepare *p*NPP reaction mixture:

2.1 Add 100 µL of distilled H2O with 0.1% BSA (H2O - 0.1% BSA) to Alkaline Phosphatase Standard (Component C, 10 units) to generate a 100 units/mLAlkaline Phosphatase standard solution.

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

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.

Table 1. 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 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

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4. Preparation of working solutions

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 pNPP working solution.

5. Run alkaline phosphatase assay in supernatants:

5.1 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.

5.2 Add 50 µL pNPP 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.

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

5.4 Monitor the absorbance increase at 400 nm using an absorbance plate reader.

6. Run alkaline phosphatase assay in cells:

6.1 Treat your cells as desired.

6.2 Add equal volume of pNPP working solution into each cell well (such as 100µL/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 2 O. 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.

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

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

Data Analysis:

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.
The typical data are shown in **Figure 1** (alkaline phosphatase standard curve).

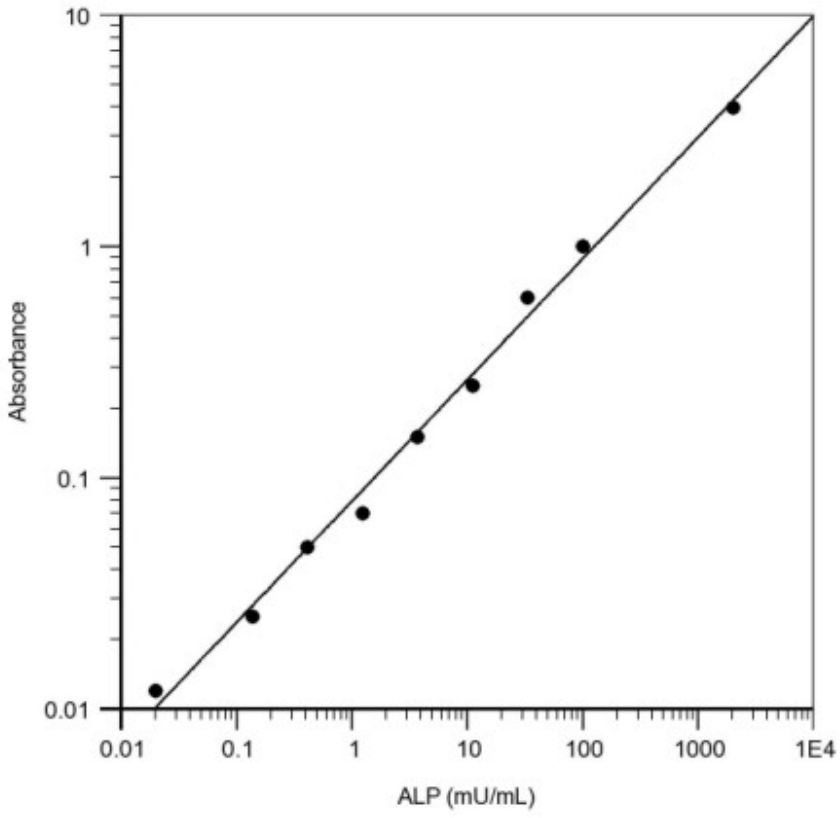

Figure 1. Alkaline phosphatase dose response was measured with the Colorimetric Alkaline Phosphatase Assay Kit in a white/clear bottom 96-well plate using a NovoStar microplate reader (BMG Labtech).

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- **Ali AT**, *et al*. Alkaline phosphatase is involved in the control of adipogenesis in the murine preadipocyte cell line, 3T3-L1. *Clin Chim Acta*, 354, 101 (2005)
- **Lee DH**, *et al*. Effects of hydrogen peroxide (H(2)O(2)) on alkaline phosphatase activity and matrix mineralization of odontoblast and osteoblast cell lines. *Cell Biol Toxicol*, 22, 39 (2006)
- **Palermo C**, *et al*. Potentiating role of IGFBP-2 on IGF-II-stimulated alkaline phosphatase activity in differentiating osteoblasts. *Am J Physiol Endocrinol Metab*, 286, E648 (2004)
- **Rieu JP**, *et al*. Insertion of GPIanchored alkaline phosphatase into supported membranes: a combined AFM and fluorescence microscopy study. *Acta Biochim Pol*, 51, 189 (2004)
- **Zhu X, Jiang C.** 8-Quinolyl phosphate as a substrate for the fluorimetric determination of alkaline phosphatase. *Clin Chim Acta.* (2006)

Technical and scientific information

Related products

- pNPP tablets, 732500
- FDP, FP-72573A
- Fluorimetric Alkaline Phosphatase Assay Kit, Blue, JQ6730
- Fluorimetric Alkaline Phosphatase Assay Kit, Green, JQ6740
- Fluorimetric Alkaline Phosphatase Assay Kit, Red, JQ6750

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
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