pNPP Phosphatase Assay

Cat. No. 8108
500 tests

Introduction

Protein phosphatase, enzyme that control the removal of phosphate (PO_4^{3-}) group from protein molecules, regulates many fundamental cellular process such as cell attachment, proliferation, differentiation and apoptosis. ScienCell™ pNPP Phosphatase Assay is optimized to detect phosphatase activity in biological samples using pNPP (4-nitrophenyl phosphate) as a colorimetric substrate for most phosphatases. A water soluble yellow product with a strong absorption at 405 nm is developed during the reaction of pNPP with phosphatase and can be detected with an ELISA plate reader.

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8108a	1	10× pNPP stock	2.5 ml	-20ºC, in the dark
8108b	1	Assay Buffer-acidic	25 ml	4ºC
8108c	1	Assay Buffer-neutral	25 ml	4ºC
8108d	1	Assay Buffer-alkaline	25 ml	4ºC
8108e	1	Stop Buffer	25 ml	4ºC

Quality Control

Phosphatase, acid from potato (Sigma cat. P1146) and alkaline from bovine intestinal mucosa (Sigma cat. P6772) were serially diluted in Assay Buffer-acidic and Assay Buffer-alkaline respectively. The activity of both phosphatases were measured with ScienCell™ pNPP Phosphatase Assay after a given time of reaction (15 min and 45 min), as shown in Figure 1 and 2.

Procedures (96-well plate)

A. Preparation of phosphatase sample

1. Prepare serial dilutions of phosphatase samples using the appropriate Assay Buffer with compatible pH (i.e.: Assay Buffer-acidic for acidic phosphatases, Assay Buffer-neutral for most neutral phosphatases, and Assay Buffer-alkaline for alkaline phosphatases).
2. We recommend a concentration range of 0-100 µg/ml for the phosphatase dilutions. Phosphatases with very high or low activity may require use of lower or higher concentrations to ensure that the absorbance reading is in the linear range.
3. A standard curve of phosphatase with known activity can be established.

B. Assay procedure

1. Apply 45 µl of each phosphate solution and 5 µl of 10× pNPP stock t to each well of the 96-well plate. Mix well and incubate for the desired period of time (10-60 minutes) at 37ºC.
2. Stop the reaction by adding 50 µl of Stop Buffer to each well, mix well and measure the absorbance on an ELISA plate reader with a test wavelength at 405 nm and a reference wavelength at 630 nm, and subtract the 630 nm background absorbance from the 405 nm measurement.

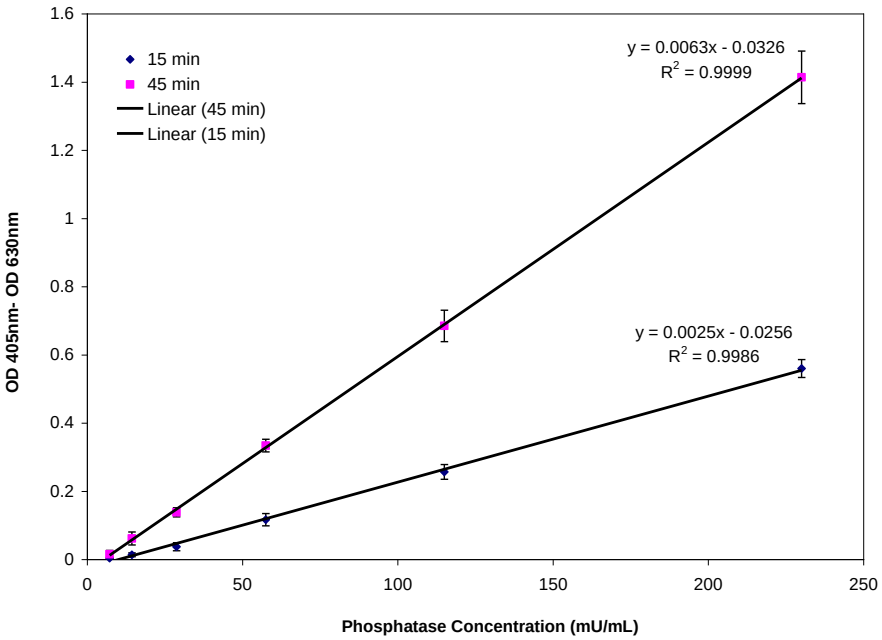

Figure 1. Phosphatase, acid from potato was serially diluted in Assay Buffer-acidic, and its activity was measured with ScienCell™ pNPP Phosphatase Assay after a given time of reaction (15 min and 45 min).

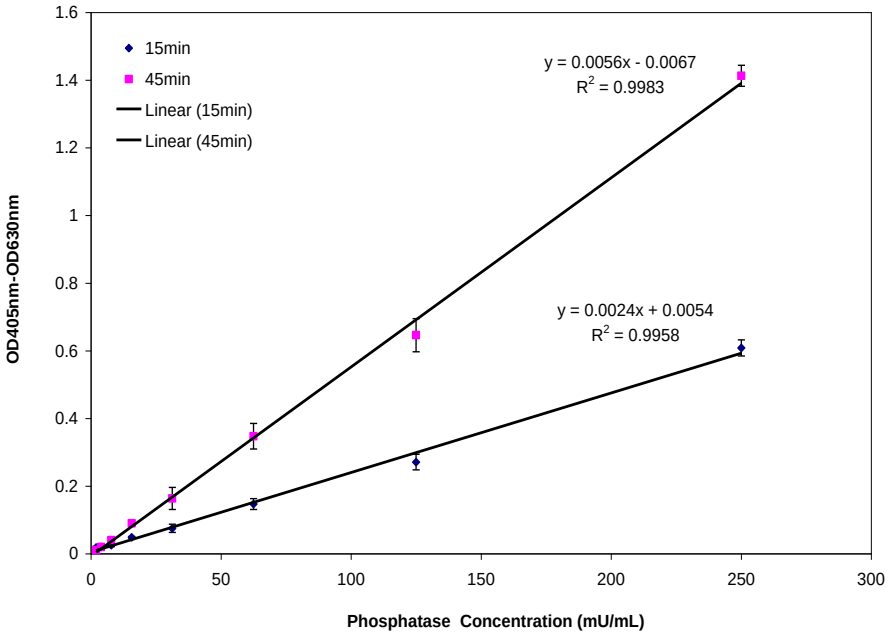

Figure 2. Phosphatase, alkaline from bovine intestinal mucosa was serially diluted in Assay Buffer-alkaline, and its activity was measured with ScienCell™ pNPP Phosphatase Assay after a given time of reaction (15 min and 45 min).