Rev. 1

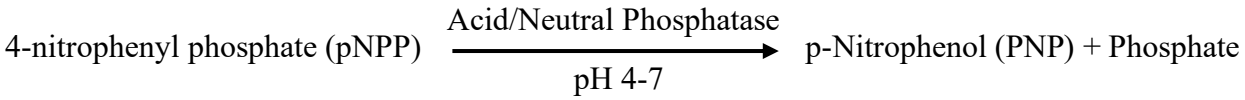
pNPP Phosphatase Assay (PNPP)

Catalog #8108

500 tests in 96-well plate

Product Description

Protein phosphatase, an enzyme that catalyzes the removal of phosphate (PO_4^{3-}) groups from protein molecules, plays a crucial role in regulating essential cellular processes such as cell attachment, proliferation, differentiation, and apoptosis. The ScienCell™ pNPP Phosphatase Assay (PNPP) is designed to detect phosphatase activity in biological samples, utilizing para-nitrophenyl phosphate (pNPP) as a chromogenic substrate. Upon hydrolysis, pNPP yields para-nitrophenol, which forms a vibrant yellow solution under alkaline conditions. This colorimetric change can be accurately measured using a spectrophotometer at a wavelength of 405 nm. The enzyme provided in the kit is an acid phosphatase derived from potato.



Sensitivity Range

1 mU/mL - 150 mU/mL

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8108a	1	Assay Buffer-acidic	25 mL	4ºC
8108b	1	Assay Buffer-neutral	25 mL	4ºC
8108c	1	Substrate	2.5 mL	-20ºC, in the dark
8108d	1	Cell Lysis Buffer	25 mL	4ºC
8108e	1	Stop Buffer	25 mL	4ºC
8108f	1	Enzyme (Lyophilized)	1 unit	-20ºC
8108g	1	Reconstitution Solution	100 µL	4ºC

Additional Materials Required (Not provided):

Microplate reader
96-well plates with clear flat-bottom
Dulbecco's Phosphate-Buffered Saline (Cat. #0303)

Quality Control

The activity of serially diluted Enzyme, Acid Phosphatase from potato, (cat #8108f) in Assay Buffer-acidic (cat #8108a) was measured with PNPP after 15-45 minutes as shown in Figure 1.

Product Use

PNPP is suitable to detect acid and neutral phosphatase activity. The Assay Buffer-acidic (Cat #8108a), with a pH of 5.5, is optimized for acid phosphatase activity. The Assay Buffer- neutral (Cat #8108b), with a pH of 7.2, is compatible with most neutral phosphatases, such as protein phosphatases. PNPP is used to evaluate phosphatase activity *in vitro* and is for research use only. It is not approved for human

or animal use or for applications in clinical or *in vitro* diagnostic procedures.

Shipping and Storage
Kit components are shipped on dry ice. Upon receipt store Cat #8108a, 8108b, 8108d, 8108e and 8108g at 4ºC and store Cat #8108c and 8108f at -20ºC in the dark.

Procedure:
Reagent Preparation

1. **Assay Buffer**: Equilibrate to room temperature before use.
2. **Enzyme Reconstitution**: Reconstitute Enzyme (cat #8108f) with 100 µL of Reconstitution Solution to make 10 U/mL enzyme stock.
3. **Standard Preparation:** Always prepare a fresh set of standards for every use.
4. **Substrate**: Use 5 µl of substrate per well of 96-well pate. Store aliquots in dark at -20°C.
5. **Stop Solution**: Equilibrate to room temperature before use.
6. **Lysis Buffer**: Ready to use. Equilibrate to room temperature before use.

Sample Preparation

1. Gently aspirate the cell culture medium (cell number may vary depending on the cell-type)
2. Wash the cells twice with Dulbecco's Phosphate-Buffered Saline.
3. Lyse the cells with appropriate amount of cell lysis buffer (0.5 ml for 35 mm dish).
4. Centrifuge the cell lysate at 14,000g for 5 minutes at 4ºC. Transfer the supernatant to a new tube.
5. Perform protein assay to determine total protein concentration in the lysate.
6. We recommend performing several dilutions in assay buffer to ensure your readings fall within the standard range.

Standard Preparation

1. Add 5 µl of Enzyme to 195 µl of Acidic assay buffer to make a 200 µl solution of 250 mU/ml enzyme standard.
2. Obtain 7 test tubes, add 150 µl of assay buffer into each tube and label them #1 through #7.
3. Add 150 µl of the 250 mU/ml enzyme standard into tube #1 and mix well to get the 125 mU/ml standard.
4. Transfer 150 µl of the 125 mU/ml enzyme standard from tube #1 to tube #2 and mix well to get the 62.5 mU/ml of standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the enzyme standards. Do not add any enzyme to tube #7, which serves as the blank.

#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**
125 mU/ml	62.5 mU/ml	31.8 mU/ml	15.6 mU/ml	7.8 mU/ml	3.9 mU/ml	Blank

Assay Procedure

1. Add 45 µl of each Standard/Sample/Blank in triplicate to each of a 96-well plate.
2. Add 5 µl of substrate per well and incubate in dark for 15-60 minutes.
3. Stop the reaction by adding 50 µl of stop buffer to each well.

Note: By adding a stop solution (alkaline) to the acidic buffer, the PNP generated in phosphatase reaction, turns yellow.

4. Mix and measure the absorbance on Microplate reader with a test wavelength at 405 and reference wave length at 630 nm. Determine the final absorbance value by subtracting the 630 nm reference absorbance from the 405 nm measurement. Example results are shown below in Figure 1.

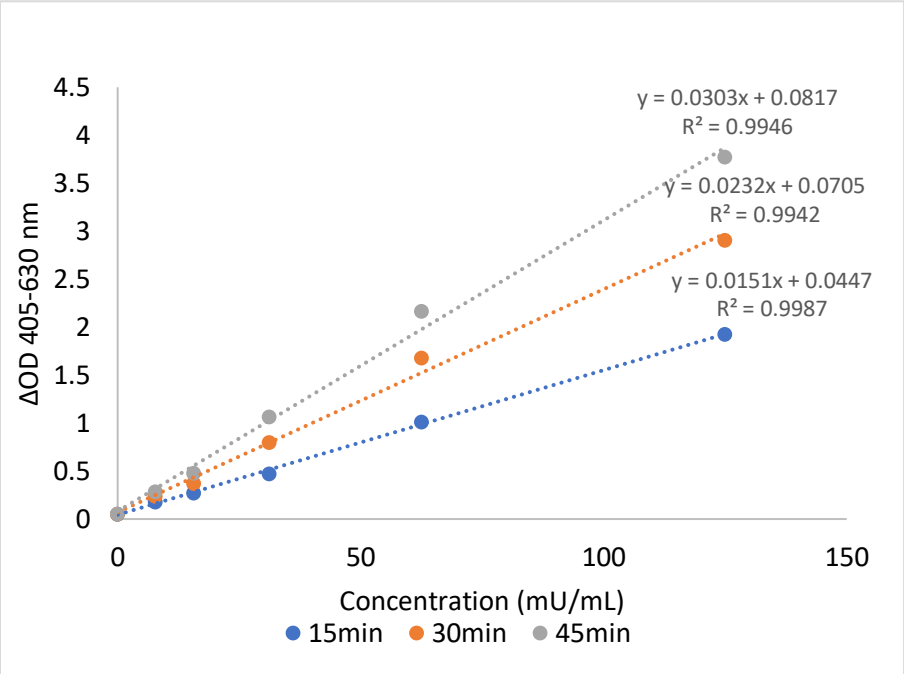


Figure 1. Enzyme was serially diluted in Assay Buffer-acidic, and its activity was measured with PNPP after a given time of reaction (15, 30 and 60 minutes).