pNPP Phosphatase Assay Kits

Colorimetric Determination of Phosphatase Activity at 405 nm

DESCRIPTION

Para-nitrophenyl phosphate (pNPP) is a chromogenic substrate for most phosphatases such as alkaline phosphatases, acid phosphatases, protein tyrosine phosphatases and serine/threonine phosphatases. The reaction yields *para*-nitrophenol, which becomes an intense yellow soluble product under alkaline conditions and can be conveniently measured at 405 nm on a spectrophotometer.

p-Nitrophenyl phosphate $\xrightarrow{\text{phosphatase}}$ *p*-nitrophenol + phosphate

This homogeneous "mix-and-measure" assay involves simply adding a single reagent to the phosphatase and measuring the product formation on an absorbance reader. The assay can be conveniently performed in cuvettes, tubes or multi-well plates at either room temperature or 37°C.

KEY FEATURES

High sensitivity and wide linear range. The detection limit is generally 3 ng phosphatase or below.
Homogeneous and simple procedure. No wash or reagent transfer steps are involved. The assay can be completed within 30 minutes.
Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

APPLICATIONS

Enzyme Activity Assay and Quality Control for phosphatase production.
Characterization of Kinetics of phosphatase reaction.
Drug Discovery: high-throughput screen for phosphatase inhibitors.

KIT CONTENTS

Catalog #	Size (assays)	Reagent	Assay Buffer	Stop Solution
POPN-500	500	280 µL	25 mL	25 mL
POPN-01K	1,000	550 µL	50 mL	50 mL
POPN-HTS	>10k	customized	customized	customized

Storage conditions. The kit is shipped at ambient temperature. Store Reagent at -20°C and other components at 4°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE USING 96-WELL PLATE

1. Equilibrate all reagents to room temperature by allowing them to stand for 30 minutes at room temperature. Prepare enough pNPP Substrate by mixing per assay 0.5 µL Reagent and 50 µL Assay Buffer.

2. Serially dilute enzyme in a proper Enzyme Buffer. Prepare enough solution for triplicate assays. Transfer 50 µL of each enzyme dilution to wells of a clear, flat-bottom 96-well plate. In addition, prepare a blank control that contains 50 µL Enzyme Buffer only. Initiate the reaction by adding 50 µL pNPP Substrate to each well.

3. Incubate for 10-30 minutes at room temperature.

4. Stop the reaction by adding 50 µL Stop Solution. Mix by quickly tapping the plate. Alternatively, plates can be shaken for 10 seconds on an orbital plate shaker.

5. Read the absorbance of each well at 405 nm.

PROCEDURE USING 384-WELL PLATE

The 384-well assay procedure is the same as for the 96-well plate protocol except that 25 µL is mixed with 25 µL pNPP Substrate. After the incubation, add 25 µL Stop Solution.

GENERAL CONSIDERATIONS

(1). Fresh reconstitution of the Reagent is recommended although the reconstituted pNPP Substrate may be stable for up to 4 weeks when stored at -20 °C. (2). Assays can be performed at room temperature or at 37 °C. (3). The pH of the Assay Buffer is 7.2 and is compatible with the majority of neutral phosphatases such as protein phosphatases. For an acid phosphatase, we recommend using 100 mM sodium acetate (pH 5.5), 10 mM $MgCl_2$ as Enzyme Buffer. For an alkaline phosphatase, we recommend using our DALP-250 Assay Kit.

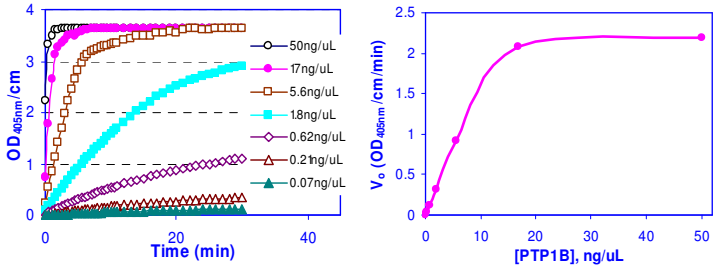
DATA ANALYSIS

Calculate the average and standard derivations of the triplicate assays and subtract the blank values. Enzyme activity is calculated from Beer-Lambert law as follows,

$$\text{Enzyme activity (µmoles/min/µg)} = \frac{\text{V (µL)} \times \text{OD}_{405nm}\ (\text{cm}^{-1})}{\varepsilon \times \text{incubation time (min)} \times \text{enzyme (µg)}}$$

$$\text{Enzyme turn-over number (min}^{-1}\text{)} = \text{Enzyme activity (µmoles/min/µg)} \times \text{enzyme molecular weight (dalton)}$$

where ε is the molar extinction coefficient ($M^{-1} \cdot cm^{-1}$). For *p*-nitrophenol, ε = $1.78 \times 10^4\ M^{-1} \cdot cm^{-1}$. OD_{405nm} (cm^{-1}) is the absorbance at 405 nm divided by the light-path length (cm). V is the final assay volume, i.e., 150 µL for 96-well plate assay and 75 µL for 384-well plate assay.



Left: PTP1B is one member of the large protein tyrosine phosphatase family. The pNPP assay was performed in a 384-well plate. After the reaction was initiated by the addition of pNPP Substrate, the plate was read on every 0.5 minutes for 30 minutes.

Right: a plot of the initial rate (V_o) against enzyme concentration. The detection limit was 3 ng PTP1B. The enzyme activity measured from the linear range was 2.2 µmoles/min/µg. The turn-over number was 82,280 per minute.

PUBLICATIONS

1. Monick, M.M. et al (2006). Active ERK contributes to protein translation by preventing JNK-dependent inhibition of protein phosphatase. J. Immunol. 177: 1636–1645.

2. Nakano, Y. (2007). Novel function of DUSP14/MKP6 (dual specific phosphatase 14) as a nonspecific regulatory molecule for delayed-type hypersensitivity. British J. Dermatology 156 (5): 848–860.

3. Lee, S.W. et al (2008). The *Xanthomonas oryzae* pv. oryzae PhoPQ two-component system is required for AvrXA21 activity, *hrpG* expression, and virulence. J. Bacteriol.190(6):2183-97.