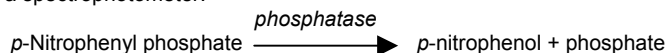


pNPP Phosphatase Assay Kits

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.



This homogeneous "mix-and-measure" assay involves simply adding a single reagent to the phosphatase and measuring the product formation using any absorbance reader. The assay can be conveniently performed in cuvettes, tubes or multi-well plates at either room temperature or 37°C. In addition, the reagents are compatible with ELISA assays in which alkaline phosphatase conjugated secondary antibody is used. This kit is well adapted for a number of applications. For example, it can be utilized for direct characterization of enzyme activity and for assay condition optimization. It can also be applied for research diagnosis of diseases that are associated with increased levels of alkaline phosphatase. Typical diseases include liver disease, bone disease, Hodgkin's disease, congestive heart failure, Fanconi's syndrome, hyperparathyroidism, intestinal disease and abdominal bacterial infections. Moreover, it can be used to characterize phosphatase inhibitors through high-throughput screening.

The kit reagents have been optimized for long shelf life and maximum reproducibility. The reagents are compatible with all liquid handling systems and bulk reagents are available for high-throughput screening of phosphatase inhibitors.

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
Z5030009	500	solid	25 mL	25 mL
Z5030010	1,000	solid	50 mL	50 mL
Z5030011	2,400	solid	120 mL	120 mL

Storage conditions. The Reagent should be stored in the amber tube at -20°C. The Assay Buffer and Stop Solution are stable at 4°C and room temperature, respectively. Shelf life: 12 months.

This protocol can be downloaded online at www.biochain.com.

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.

PROCEDURES

Procedure using 96-well plate:

1. Equilibrate Assay Buffer and Stop Solution to room temperature by allowing them to stand for 30 minutes at room temperature. Reconstitute the Reagent with the provided Assay Buffer. Simply combine the Assay Buffer and Reagent by pipetting a small volume (e.g. 1 mL) buffer to the Reagent tube. Vortex briefly and transfer the reconstituted solution to the Assay Buffer bottle. Repeat this step to transfer all Reagent to the Assay

Buffer bottle. Mix by inversion until the Reagent is thoroughly dissolved. After this is done, mark the bottle label as Reconstituted Reagent.

2. Series dilute enzyme in a proper Enzyme Buffer. Prepare enough solution for triplicate assays. Transfer 50 µL of each enzyme dilution to wells in the test plate. In addition, prepare blank wells that contain 50 µL Enzyme Buffer without any enzyme. Initiate the reaction by adding 50 µL pNPP substrate solution.

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:

1. Equilibrate Assay Buffer and Stop Solution to room temperature by allowing them to stand for 30 minutes at room temperature. Reconstitute the Reagent with the provided Assay Buffer. Simply combine the Assay Buffer and Reagent by pipetting a small volume (e.g. 1 mL) buffer to the Reagent tube. Vortex briefly and transfer the reconstituted solution to the Assay Buffer bottle. Repeat this step to transfer all reagents to the Assay Buffer bottle. Mix by inversion until the Reagent is thoroughly dissolved. After this is done, mark the bottle label as Reconstituted Reagent.

2. Series dilute enzyme in a proper Enzyme Buffer. Prepare enough solution for triplicate assays. Transfer 25 µL of each enzyme dilution to wells in the test plate. In addition, prepare blank wells that contain 25 µL Enzyme Buffer without any enzyme. Initiate the reaction by adding 25 µL pNPP substrate solution.

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

4. Stop reaction by adding 25 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.

GENERAL CONSIDERATIONS

(1). Fresh reconstitution of the Reagent is recommended although the reconstituted reagent 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 phosphatases. For an acid phosphatase, we recommend using 100mM sodium acetate (pH 5.5), 10mM MgCl₂ as Enzyme Buffer. For an alkaline phosphatase, we recommend using 100mM Tris-HCl (pH 8.6), 10mM MgCl₂ as Enzyme Buffer.

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,

$$\text{Enzyme activity } (\mu\text{moles/min}/\mu\text{g}) = \frac{V (\mu\text{L}) \times \text{OD}_{405\text{nm}} (\text{cm}^{-1})}{\epsilon \times \text{incubation time (min)} \times \text{enzyme } (\mu\text{g})}$$

$$\text{Enzyme turn-over number (min}^{-1}\text{)} = \frac{\text{Enzyme activity } (\mu\text{moles/min}/\mu\text{g})}{\text{enzyme molecular weight (dalton)}}$$

where ϵ is the molar extinction coefficient ($\text{M}^{-1} \cdot \text{cm}^{-1}$). For *p*-nitrophenol, $\epsilon = 1.78 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$. $\text{OD}_{405\text{nm}} (\text{cm}^{-1})$ 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.

LITERATURE

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TECHNICAL NOTES

The pNPP assay kits have been specially optimized and formulated to provide a convenient and sensitive assay for a large number of phosphatases. Key features of the kits are as follows:

Good 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.

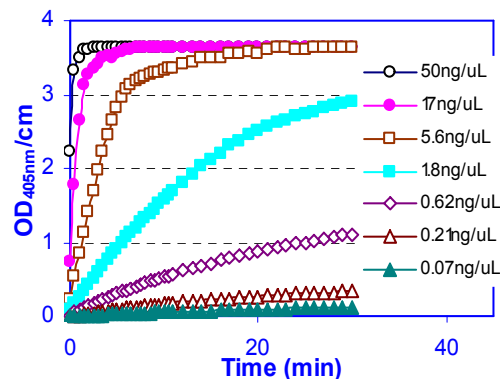


Figure 1. PTP1B is one member of the large protein tyrosine phosphatase family. The pNPP assay was performed according to the standard protocol for 384-well microtiter plate assay. Immediately after the reaction was initiated by the addition of pNPP, the plate was read on a SPECRTAmax384 PLUS (Molecular Devices) every 0.5 minutes for 30 minutes.

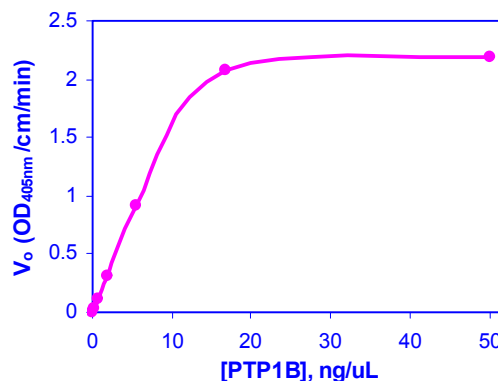


Figure 2. A plot of the initial rate (V_0) against enzyme concentration. The detection limit was 3 ng PTP1B. The enzyme activity measured from the linear range was 2.2 $\mu\text{moles}/\text{min}/\mu\text{g}$. The turn-over number was 82,280 per minute. Data calculated from Figure 1.



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