Technical Bulletin

pNPP Phosphatase Assay Kit

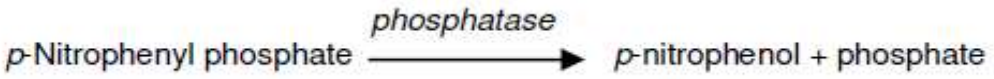
Catalog Number MAK461

Product 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.

The pNPP Phosphatase Assay kit involves adding a single reagent to the phosphatase and measuring the product formation on a spectrophotometer. The assay can be conveniently performed in cuvettes, tubes or multiwell plates at either room temperature or 37 °C. The detection limit of the kit is generally 3 ng of phosphatase or below.

The kit is suitable for the quantitative determination of phosphatase activity of protein phosphatases.



Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- Reagent 280 µL
  Catalog Number MAK461A
- Assay Buffer 25 mL
  Catalog Number MAK461B
- Stop Solution 25 mL
  Catalog Number MAK461C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader which measures absorbance per cm
- Clear flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- (Optional) *p*-Nitrophenol for use with plate readers that do not measure absorbance per cm (Catalog Number 1048 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store Reagent at -20 °C and other components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Prior to assay, allow all reagents to equilibrate to room temperature for 30 minutes.

Procedure

All samples should be run in triplicate.

96-well Procedure

1. Mix enough reagents for the number of assays to be performed. For each well, prepare pNPP Substrate according to Table 1. Prepare pNPP Substrate fresh just prior to assay.

   Table 1.
   Preparation of pNPP Substrate

Reagent	pNPP Substrate
Assay Buffer	50 µL
Reagent	0.5 µL

2. Prepare serial dilutions of the Sample Enzyme in an appropriate enzyme buffer. Prepare enough solution for triplicate assays.

   Note: The pH of the Assay Buffer provided in the kit is 7.2 and is compatible with the majority of neutral phosphatases such as protein phosphatases.

   For an acid phosphatase, use 100 mM sodium acetate, pH 5.5, 10 mM $MgCl_2$ as the enzyme buffer.

   For an alkaline phosphatase, assay using the Alkaline Phosphatase Assay Kit (Catalog Number MAK447).

3. Transfer 50 µL of each Sample Enzyme dilution to wells of a clear, flat-bottom 96-well plate.
4. In triplicate, prepare Blank Control wells that contain 50 µL of enzyme buffer.
5. Initiate the reaction by adding 50 µL of pNPP Substrate to each well.
6. Incubate for 10-30 minutes at room temperature or 37 °C.
7. Stop the reaction by adding 50 µL of Stop Solution. Mix by quickly tapping the plate. Alternatively, plates can be shaken for 10 seconds on an orbital plate shaker.
8. Read the absorbance of each well at 405 nm (A_{405}).

   Note: If using a plate reader which does not measure absorbance per cm, the light pathlength for the reaction can be determined separately using a dye with known concentration and molar extinction coefficient, e.g., *p*-nitrophenol in the Assay Buffer.

384-well Procedure

Follow the 96-well Procedure except adjust the volumes added to the plate to 25 µL Sample Enzyme and 25 µL pNPP Substrate. After the incubation, add 25 µL Stop Solution.

Results

1. For each Sample Enzyme serial dilution, calculate the average absorbance (A_{405}) and standard derivations of the triplicate wells.
2. Subtract the average Blank Control absorbance (A_{405}) from each Sample Enzyme serial dilution absorbance (A_{405}) to determine the corrected Sample Enzyme absorbance (ΔA_{405}).
3. The enzyme activity is calculated from Beer-Lambert law as follows:

Enzyme activity (μmoles/min/μg) =

$$\frac{V\ (\mu l)\ x\ \Delta A_{405}\ (cm^{-1})}{\varepsilon\ x\ T\ (min)\ x\ enzyme\ (\mu g)}$$

where

ε = Molar extinction coefficient ($M^{-1} \times cm^{-1}$). For *p*-nitrophenol, $\varepsilon = 1.78 \times 10^4\ M^{-1} \times cm^{-1}$.

ΔA_{405} = Absorbance at 405 nm divided by the light path length (cm)

V = Final assay volume (150 μL for 96-well plate assay and 75 μL for 384-well plate assay)

T = Reaction time (minutes) (See Step 6 of the procedure)

Enzyme= Amount of Sample Enzyme added to the reaction (μg)

Enzyme turn-over number (min^{-1}) =

Enzyme activity (μmoles/min/μg) × Enzyme molecular weight (Dalton)

Figure 1.
PTP1B is one member of the large protein tyrosine phosphatase family. The pNPP assay was performed in a 384-well plate using serial dilutions of the PTP1B enzyme. After the reaction was initiated by the addition of pNPP Substrate, the plate was read every 30 seconds for 30 minutes.

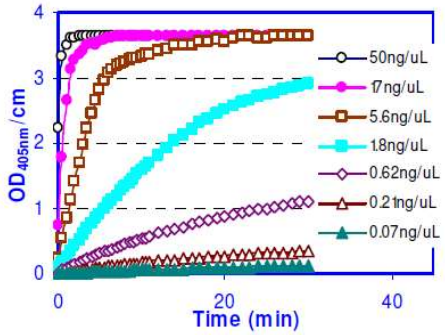
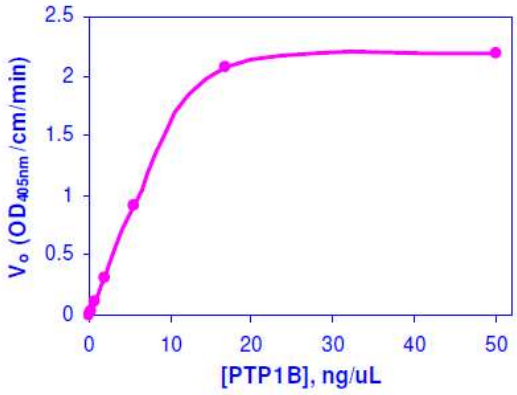


Figure 2.
Plot of the initial rate (V_o) against enzyme concentration. The detection limit was 3 ng PTP1B. The enzyme activity measured from the linear range was 2.2 μmoles/min/μg. The turn-over number was 82,280 per minute.



References

1. Lopez-Vales, R., et al., Fenretinide promotes functional recovery and tissue protection after spinal cord contusion injury in mice. *J. Neurosci*., **30**, 3220-6 (2010).
2. Oborna, I., et al., Increased lipid peroxidation and abnormal fatty acid profiles in seminal and blood plasma of normozoospermic males from infertile couples. *Hum. Reprod*., **25**, 308-16 (2010).
3. Olsen, A.S., et al., Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. *Wound Repair Regen*., **18**, 532-42 (2010).

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