Cat.-No.	Size
PR-944	1 Kit

For *in vitro* use only
Quality guaranteed for 12 months
DTT and dissolved pNPP should be stored at -20°C. Buffers, pNPP tablets should be stored at 4°C.
Protect pNPP from light.
EDTA, NaCl and Stop Solution can be stored at room temperature.

Description

Protein phosphatases regulate fundamental biological processes such as cell proliferation and differentiation in cooperation with protein kinases. Protein phosphatases consist of three general families with different substrate specificities towards phospho-seryl/threonyl, -tyrosyl residues, or both.
This Kit provides a simple method to measure phosphatase activity by using pNPP as a general substrate of phosphatases.

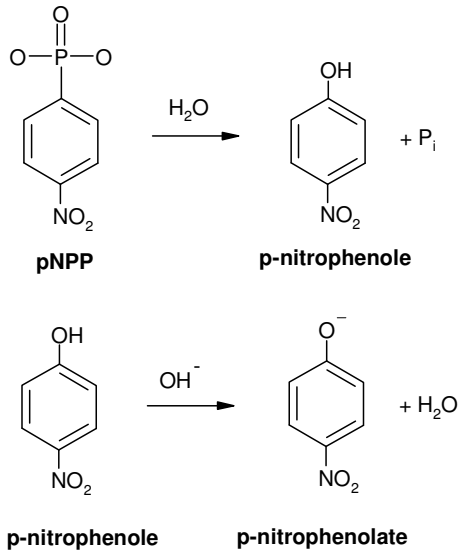

Fig. 1: In a first step, the enzyme dephosphorylates its substrate pNPP. In a second step, the phenolic OH-group is deprotonated under alkaline conditions resulting in p-nitrophenolate with a strong absorption at 405 nm (yellow).

Kit Contents

- ✓ 1 ml MES Buffer **1** (1 M, pH 5.5)
- ✓ 1 ml MES Buffer **2** (1 M, pH 6.0)
- ✓ 1 ml MES Buffer **3** (1 M, pH 6.5)
- ✓ 1 ml HEPES Buffer **4** (1 M, pH 7.0)
- ✓ 1 ml HEPES Buffer **5** (1 M, pH 7.5)
- ✓ 1 ml HEPES Buffer **6** (1 M, pH 8.0)
- ✓ 1 ml NaCl (1.5 M)
- ✓ 1 ml EDTA (10 mM, pH 8.2)
- ✓ 1 ml DTT (50 mM)
- ✓ 10 ml Stop Solution (1 N NaOH)
- ✓ 5x pNPP Substrate Tablets (5 mg each)
- ✓ 1 Microtiter Plate (96 well)

Additional Reagents and Material required

- Microtiter Plate Reader (wavelength 405 nm)
- Microcentrifuge Vials
- Ultrapure Water

Recommended PCR assay

1) Dissolve 1 tablet pNPP in 188 µl ultrapure water to prepare a 100 mM = 10x stock solution. Avoid touching the substrate tablets with hands!

   Please note: The prepared pNPP solution can be stored at -20°C. However, the preparation of a fresh stock for each usage is recommend.

2) Prepare 1 ml of reaction buffer by combining the 10x stocks:
   100 µl Hepes Buffer 4 (pH 7.0) *
   100 µl NaCl
   100 µl EDTA
   100 µl DTT
   600 µl ultrapure water

* Hepes Buffer 4 (pH 7.0) is a standard buffer close to physiological pH. Your particular phosphatase may require one of the other buffers provided for maximum activity.

3) Prepare 10 µl of a solution of the phosphatase with a concentration of approx. 0.1 mg/ml in reaction buffer. This concentration corresponds to a typical assay concentration of 100 ng phosphatase per µl assay volume. Keep in mind that phosphatases with very high/low activities may require the use of less/more enzyme, respectively.

4) Prepare 12 microcentrifuge tubes. Use 2 x 4 tubes for the phosphatase reaction (e.g. 5 min, 10 min, 30 min and 60 min incubation in duplets) and 4 tubes for background correction of intrinsic pNPP absorption.

5) Add 44 µl reaction buffer and 1 µl of phosphatase to each of the "phosphatise reaction" tubes. Add 45 µl reaction buffer to each of the four "background correction" tubes.

6) Start the reactions by adding 5 µl of 10x pNPP solution from Step 1 to each tube and vortex gently.

7) Place the tubes in a thermoblock and incubate at 30°C.

8) Stop the reactions (2 samples and one blank) after the required times (e.g. 5 min, 10 min, 30 min and 60 min) with 2 volumes (100 µl) of Stop Solution (1N NaOH).

9) Pipette the samples into the Microtiter Plate and measure extinction (OD) at 405 nm.

Calculation of Enzyme Activity

Phosphatase activity is calculated according to Lambert-Beer's law as following:

$$A = \frac{E_{405} \cdot V_{total}}{t \cdot \varepsilon \cdot d \cdot m_{Enzyme}}$$

A: phosphatase activity [nmol P_i per min per µg]
E_{405}: extinction (OD) of the sample at 405 nm
V_{total}: total sample volume
[reaction volume + stop solution = 150 µl]
t: reaction time [min]
ε: extinction coefficient [17.8 µl · $nmol^{-1}$ · cm^{-1}]
d: pathlength of light [here: 0.6 cm]
m_{Enzyme}: amount of enzyme [µg]

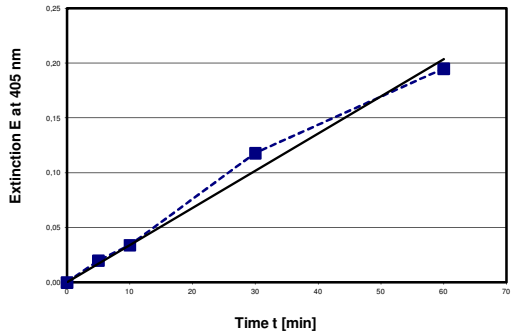

Fig. 2: Phosphatase SHP-1 (100 ng) was assayed with 10 mM pNPP in JBS Phosphatase Assay. This type of assay is called "end-point" assay since the activity of the enzyme is evaluated after a given time of reaction (here: 5, 10, 30 and 60 min).

Comment

The standard assay conditions described above are recommendations. In some cases it may be necessary to increase the amount of pNPP (up to 50 mM final concentration), to perform the assay at different temperatures (such as 37°C) or to modify the reaction buffer to meet specific needs of a particular enzyme.

Selected References

Voller *et al.* (1976) Enzyme immunoassays in diagnostic medicine. Theory and practice. Bull. *World Health Organ* **53**:55.

Engvall E. (1980) Enzyme immunoassay ELISA and EMIT. *Methods Enzymol.* **70**:419.

Sirois *et al.* (1984) An enzyme-linked immunosorbent assay for the detection of complement components on red blood cells. Am. *J. Clin. Pathol.* **82**:67.

Frank *et al.* (1999) Binding of phosphatidic acid to the protein-tyrosine phosphatase SHP-1 as a basis for activity modulation. *Biochemistry* 38:11993.

Rigby *et al.* (2011) Roles of biogenic amines in regulating bioluminescence in the Australian glowworm Arachnocampa flava. *The Journal of Experimental Biology* **214(19)**:3286.