The assay can be used for the quick analysis of the protein phosphatase activity under any non-standard conditions.

The advantage of the PNPP phosphatase activity assay is that unlike radioactive assays the substrate concentration can be much higher than the $K_m$. The initial velocity can be recorded in the continuous assay, but the assay volume is larger than in a radioactive assay (about 1 ml to fill a 1 ml spectrophotometer cuvette) (1,2). The reaction volume in a single-point assay can be very small because the reaction is stopped with the amount of NaOH enough to fill the cuvette (1,3,4).

**Notes on Use in Protein Phosphatase Assay:** The PNPP phosphatase activity assay is very simple, non-expensive, and routinely used for the unit determination of all NEB protein phosphatases. PNPP has apparent $K_m$ values for protein phosphatases in the range of 0.5–10 mM (2-5). The PNPP phosphatase activity is assayed in a reaction mixture (50 μl) containing 50 mM PNPP and a protein phosphatase buffer supplemented with additional components when required. The reaction is initiated by addition of enzyme and quenched after 5-10 minutes by addition of 1 ml of 1 N NaOH (or 1 ml of 0.5 M EDTA for Mn$^{2+}$-dependent protein phosphatases, λ-$PPase$ and PP1). The amount of product, p-nitrophenol, is determined by reading the absorbance at 405 nm and using a molar extinction coefficient of 18,000 M$^{-1}$ cm$^{-1}$ (16,000 M$^{-1}$ cm$^{-1}$ for 0.5 M EDTA) (1, 3).

One unit of the protein phosphatase activity is defined as the amount of enzyme that hydrolyzes 1 nanomole of PNPP in one minute at 30°C in a total reaction volume of 50 μl under standard reaction conditions.

To estimate the protein phosphatase activity accurately it is essential to ensure linear kinetics of dephosphorylation.

**References:**

**Suggested Working Concentration:**
50–100 mM

**Purity:** >99% pure.

**Molecular Weight:** 461.4 daltons [di(tris)salt].

**Supplied in:** Sterile purified water.

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