The advantage of the PNPP phosphatase activity assay is that unlike radioactive assays the substrate concentration can be much higher than the $K_v$. 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).

Supplied in: Sterile purified water.

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

Purity: >99% pure.

Suggested Working Concentration: 50–100 mM

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 $K_v$ 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 Mn²⁺-dependent protein phosphatases, X-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 condition. To estimate the protein phosphatase activity accurately it is essential to ensure linear kinetics of dephosphorylation.

References:

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