V.2.0

ELP03: Purine nucleoside phosphorylase (PNP)
(recombinant *B. subtilus* gene over-expressed in *E. coli*)
Lot:
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

The purine nucleoside phosphorylase protein has been produced in a bacterial expression system. The recombinant protein (aa1-283) contains a 6xHIS-tag at the amino terminus and has a combined calculated molecular weight of approximately 32 kDa. The protein has been determined to be biologically active in a microtubule-activated phosphate transfer assay (see below). The protein is supplied as a white lyophilized powder.

Figure 1: PNP transferase assay.

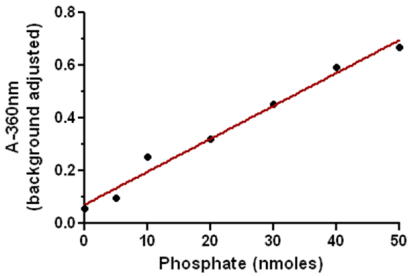


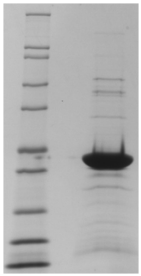
Figure 1 legend: PNP was incubated with MESG at pH 7.0 in the presence of magnesium. The reaction is able to detect down to 3 nmole of Pi in a 150 µl (20 µM).

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on an SDS gradient gel. Figure 2 shows 20 µg of ELP01 protein which was determined to be >90% pure.

Storage and Reconstitution

Shipped at ambient temperature. The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year.
Briefly centrifuge to collect the protein at the bottom of the tube. The protein should be reconstituted with 500 µl of distilled water and stored at 4°C where it will be stable for 6 months. For working concentrations dilute 100 fold into the reaction mixture.

Figure 2. PNP Protein Purity.



Legend Figure 2: Lane 1; Molecular weight markers, Lane 2; 20 µg samples of PNP protein, were separated on a 4-20% SDS PAGE gel and stained with Coomassie Blue. The PNP protein has an approximate molecular weight of 32-34 kDa. Protein quantitation was determined using the Precision Red™ Protein Assay reagent. (Cat. # ADV02).

Phosphate Transferase Assay

The assay is based upon an absorbance shift (330 nm—360 nm) that occurs when 2-amino-6-mercapto-7-methylpurine ribonucleoside (MESG) is catalytically converted to 2-amino-6-mercapto-7-methylpurine in the presence of inorganic phosphate (Pi). One molecule of Pi will yield one molecule of 2-amino-6-mercapto-7-methylpurine in an essentially irreversible reaction. Hence, in the presence of an ATPase or GTPase generating phosphate, the absorbance at 360 nm is directly proportional to the amount of Pi generated in an enzyme reaction.

Reagents

1) MESG substrate from BK051 ATPase kit.
2) Reaction buffer 15 mM Pipes-KOH or Tris-HCl pH 7.0 with 5 mM MgCl2.
3) Phosphate solution at 1 mM.

Equipment

1) Monochromatic spectrophotometer (set to 360 nm) or a filter based spectrophotometer with a 360 nm filter and bandwidth of <10nm.

Method (ELIPA ATPase Assay)

The reactions were conducted in a 96-well half-area well plate (135 µl reaction volumes). 1.5 µl of stock PNP, 0.2 mM MESG, 15 mM PIPES pH 7.0, 5 mM $MgCl_2$. Reactions are initiated by adding 15 µl of 10x concentrated phosphate in water. Control reactions were carried out in the absence of phosphate. Reactions were measured in

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a M2 plate reader (Molecular Devices) set in endpoint mode at 360 nm absorbance wavelength. The nmoles of ATP generated in a given time was determined by the use of a phosphate standard curve (Figure 1).

Product Uses

- Measurement of phosphate in buffers or serum.
- Identification/characterization of ATPase proteins.
- Measurement of enzymatic parameters of ATPases and GTPases.

References

1. Webb, M.R. 1992. A continuous spectrophotometric assay for inorganic phosphate and for measuring phosphate release kinetics in biological systems. Proc. Natl. Acad. Sci. USA 89: 4884-4887.

2. Cheng Q., Wang Z-X., and Killilea SD. 1997. A continuous spectrophotometric assay for protein phosphatases. Analytical Biochemistry 226: 68-73.

3. Funk, C.J. et al. 2004. Development of High Throughput Screens for Discovery of Kinesin ATPase modulators. Analytical Biochemistry. 329: 68-76 .