



## ProFoldin

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## INSTRUCTIONS

# ProFoldin

## Polynucleotide Phosphorylase (PNPase) Assay Kits

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*E. coli* Polynucleotide Phosphorylase (PNPase) Assay Kit

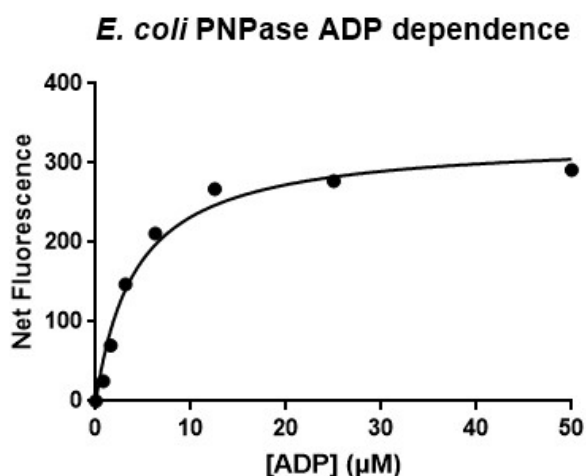
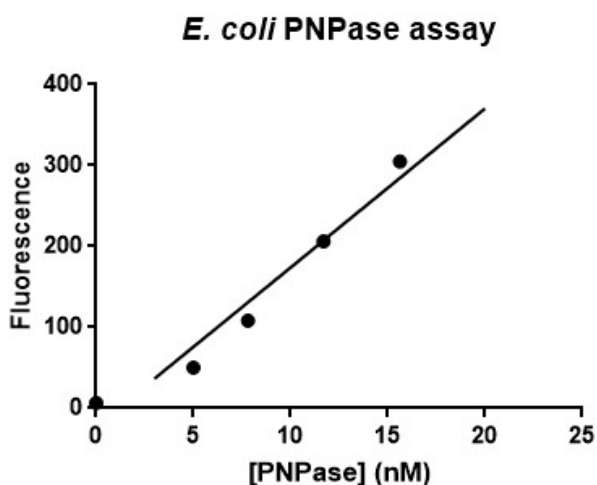
Catalog No. PNP100K

*E. coli* Polynucleotide Phosphorylase (PNPase) Assay Kit Plus

Catalog No. PNP100KE

### Introduction

Polynucleotide Phosphorylase (PNPase) plays important roles in mRNA processing and degradation in various cells. RNA metabolism in bacteria involves posttranscriptional addition of poly(A) tails. PNPase has both exonuclease and polymerase activities. The *E. coli* PNPase assay is based on measurement of Poly(A) synthesized by *E. coli* PNPase using ADP. The assay can be performed in a 384-well or 96-well plate format for tests of *E. coli* PNPase activities and high throughput screening of inhibitors.



The *E. coli* Polynucleotide Phosphorylase (PNPase) Assay Kit (Catalog No. PNP100K) includes 400 µl of 10 x Buffer, 33 µl of 100 x RNA, 33 µl of 100 x ADP (2.5 mM) and 330 µl of 10 x fluorescence dye. It is for 100 assays of *E. coli* PNPase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

The *E. coli* Polynucleotide Phosphorylase (PNPase) Assay Kit Plus (Catalog No. PNP100KE)

includes all the reagents for 100 assays of *E. coli* PNPase in a 384-well plate format. The kit includes 400 µl of 10 x Buffer, 33 µl of 100 x RNA, 33 µl of 100 x ADP (2.5 mM), 330 µl of 10 x fluorescence dye and 33 µl of 100 x *E. coli* PNPase (5 µM).

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## ASSAY PROTOCOL

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike). The reaction volume is 30  $\mu$ l and the final assay volume is 60  $\mu$ l. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60  $\mu$ l and the final assay volume is 120  $\mu$ l.

### 1. Reaction:

The total volume of each reaction mixture is 30  $\mu$ l including 26.1  $\mu$ l of H<sub>2</sub>O, 3  $\mu$ l of 10 x Buffer, 0.3  $\mu$ l of 100 x template, 0.3  $\mu$ l of 100 x PNPase and 0.3  $\mu$ l of 100 x ADP. Incubate the reaction mixture at 37°C for 60 min.

### 2. Detection:

Dilute the 10x fluorescence dye with water to make the 1 x dye. Mix 30  $\mu$ l of the 1 x dye with 30  $\mu$ l of the reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm in 5 min.

## Assay Protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

## Related Products

VRT100K	Viral RNA-dependent RNA Polymerase Assay Kit – 100
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
HCMV100K	Human Cytomegalovirus (HCMV) DNA Polymerase Assay Kit
DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	<i>H influenzae</i> DNA Polymerase Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA Polymerase Assay Kit Plus
HDP A100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
PAR100KE	Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus

For more information of drug targets and enzyme assays, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).