



## ProFoldin

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# INSTRUCTIONS

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### *S. pneumoniae* Nicotinate Phosphoribosyltransferase (PNCB) Assay Kits

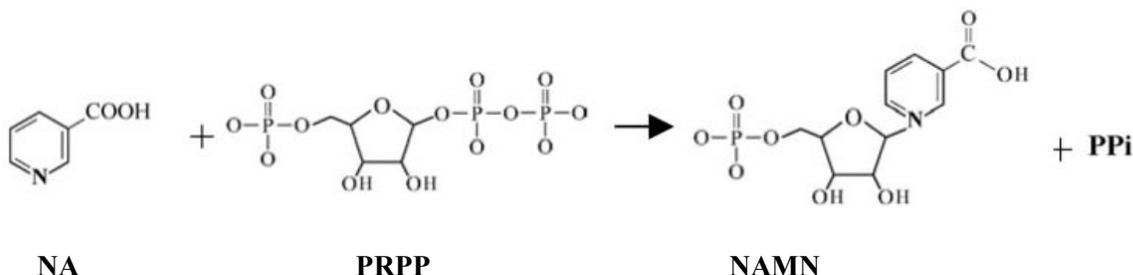
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*S. pneumoniae* PNCB Assay Kit Plus-100 Cat#. PNCB100KN

*S. pneumoniae* PNCB Assay Kit Plus-500 Cat#. PNCB500KN

#### INTRODUCTION

Nicotinate phosphoribosyltransferase (NAPRTase or PNCB) provides a pathway of regenerating nicotinic acid adenine dinucleotide (NAD) through synthesizing nicotinic acid mononucleotide (NAMN). The enzyme transfers nicotinate (NA) onto phosphoribosyl pyrophosphate (PRPP) releasing pyrophosphate. PNCB is drug target for gram (+) bacteria.



The **Nicotinate Phosphoribosyltransferase (PNCB) Assay** is based on measurement of the pyrophosphate generated from the enzyme reaction. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of PNCB in drug discovery research. It may also be used for characterization of PNCB enzyme.

The ***S. pneumoniae* PNCB Assay Kit Plus-100 (Catalog No. PNCB100KN)** contains the reagents for 100 assays in a 384-well plate assay format including 300  $\mu$ l of 10 x Buffer, 33  $\mu$ l of 100 x NA, 33  $\mu$ l of 100 x PRPP, 33  $\mu$ l of 100 x ATP, 33  $\mu$ l of 100 x *S. pneumoniae* PNCB (5000 nM), 33  $\mu$ l of 100 x pyrophosphatase (PPase, 10 U/ml) and 4 ml of Dye MPA3000 for phosphate detection.

The ***S. pneumoniae* PNCB Assay Kit Plus-500 (Catalog No. PNCB500KN)** contains the reagents for 500 assays in a 384-well plate assay format including 2000  $\mu$ l of 10 x Buffer, 170  $\mu$ l of 100 x NA, 170  $\mu$ l of 100 x PRPP, 170  $\mu$ l of 100 x ATP, 170  $\mu$ l of 100 x *S. pneumoniae* PNCB (5000 nM), 170  $\mu$ l of 100 x pyrophosphatase (PPase, 10 U/ml), and 25 ml of Dye MPA3000 for phosphate detection.

#### ASSAY PROTOCOL

The following assay protocol is based on the 384-well plate assay format. The reaction volume is 30  $\mu$ l and the final assay volume is 75  $\mu$ l. For 96-well plate assays, the reaction volume is 60  $\mu$ l and the final

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assay volume is 150  $\mu$ l. For detection using a cuvette, the reaction volume is 400  $\mu$ l and the final assay volume is 1000  $\mu$ l.

## 1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 297  $\mu$ l of premix composed of 257.4  $\mu$ l of H<sub>2</sub>O, 33  $\mu$ l of 10 x Buffer, 3.3  $\mu$ l of 100 x *S. pneumoniae* PNCB and 3.3  $\mu$ l of 100 x PPase .
- (2) Prepare 33  $\mu$ l of 10 x Enzyme substrate by mixing 3.3  $\mu$ l of 100 x NA, 3.3  $\mu$ l of 100 x PRPP, 3.3  $\mu$ l of 100 x ATP with 23.1  $\mu$ l of water.

## 2. Reaction:

Mix 27  $\mu$ l of the premix with 3  $\mu$ l of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37°C for 60 min.

## 3. Detection:

Add 45  $\mu$ l of the Dye MPA3000 into the 30  $\mu$ l of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

## ASSAY LINEARITY TEST

Follow the same protocol described above except mixing 27  $\mu$ l of the premix with 3  $\mu$ l of the 10 x Enzyme substrate at different time points. Plot the reaction signal versus the reaction time to define the linear range.

## IC50 MEASUREMENT OF ENZYME INHIBITORS

The concentration range of the inhibitor to be tested depends on the potency of the inhibitor. In general, the maximum concentration is about 10 to 20 fold higher than the IC50 value. The following protocol is for IC50 measurement of one inhibitor with IC50 values around 10  $\mu$ M.

1. In 8 assay wells, add 0.6  $\mu$ l of 2-fold serial dilution solutions of the inhibitor from 5 mM to 0.039 mM in water or DMSO. In one control well, add 0.6  $\mu$ l of water or DMSO. In another control well, add 0.6  $\mu$ l of 1 M EDTA.
2. Prepare 297  $\mu$ l of premix and 33  $\mu$ l of 10 x Enzyme substrate as described above.
3. Mix 26.4  $\mu$ l of the premix and 0.6  $\mu$ l of the 50 x inhibitor for 5 min.
4. Add 3  $\mu$ l of the 10 x Enzyme substrate and incubate the assay reaction for the time in the linear range.
5. Add 45  $\mu$ l of the Dye MPA3000 into the 30  $\mu$ l of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.
6. Calculate IC50s using a computer IC50 fitting software.

## RELATED PRODUCTS

*S. aureus* PncB Assay Kit Plus-100 (Cat#. PNCB100KS)

*S. aureus* PncB Assay Kit Plus-500 (Cat#. PNCB500KS)

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).