**Product number: Pnk162**
**Lot number:**

# ThermoPhage™ Polynucleotide Kinase

## Introduction

### Product Description

ThermoPhage™ Polynucleotide Kinase (PNK) catalyses the transfer of phosphate (Pi) from the γ-position of ATP to the 5' end of a hydroxylated nucleic acid (RNA or DNA, single or double stranded) or an exchange of the Pi to a mono-phosphorylated nucleic acid (1).

ThermoPhage™ PNK is derived from bacteriophage RM378 that infects *Rhodothermus marinus* (2). The recombinant PNK protein was expressed and purified from *E. coli*.

ThermoPhage™ PNK has a temperature optimum between 60-70°C. For short incubation time (1 hour or less) temperature optimum of the enzyme is about 70 °C but for longer incubation protocols we recommend 60-65°C.

ThermoPhage™ PNK has $Mn^{2+}$ -dependent 3' phosphohydrolase activity that does not interfere with its 5' kinase activity.

### Applications

- Labeling of nucleic acids using $^{32}$P-γ-ATP for probes and DNA sequencing.
- Phosphorylation of nucleic acids for subsequent ligation for cloning.
- Phosphorylation of oligonucleotides for ligase reaction like the Ligase Chain Reaction and similar procedures (3-4).
- Phosphorylation of nucleic acids with modified phosphates (i.e. thiol-phosphates) for subsequent modifications and/or labeling.

Notes:
The ThermoPhage™ PNK is inhibited by high (>50 mM) salt concentrations (KCl and NaCl), and also strongly inhibited by phosphate ions and ammonium ions.

Labeling of 5'phosphorylated nucleic acids (exchange reaction) will work in the same buffer as the forward reaction but more time and enzyme is needed.

If using protocols including RNA and RNAse inhibitors, make sure they are active at high temperature, for example SUPERase InTM (Ambion) or RNAse-Free Ribonuclease inhibitor (CHIMERx).

### Storage

Storage and dilution buffer: 10 mM Tris (pH 8), 50 mM KCl, 0.1 mM EDTA, 0.1 µM ATP, 1 mM DTT and 50 % glycerol. ThermoPhage™ PNK is stable for one year when stored at –25 to -15 °C.

### Reaction Conditions for unit definition

1 x reaction buffer (50 mM MOPS (pH 8.5), 1 mM DTT, 10 mM $MgCl_2$, 10 mM KCl), with 2 nmol ATP, 25 mg/ml BSA, 5% PEG6000, 1 nmol ssDNA oligonucleotides and ThermoPhage™ PNK enzyme incubated at 70°C for 15 minutes in 20 ml volume.

### Concentration and Unit Definition

Concentration 25 U/ml
One unit of ThermoPhage™ Polynucleotide Kinase catalyses the transfer of 1 nmol of γ-phosphate from ATP to 5' hydroxylated end of oligonucleotides at 70°C in 30 minutes (5).

## Application protocol

### Reaction Protocol

For optimized phosphorylation reaction protocol use 10-20 units of enzyme in a 25-50 µl reaction volume with 1x reaction buffer, 5 % PEG6000, 25 µg/ml BSA, 10-100 µM ATP and substrate. Incubate at 60-70°C for 1-2 hours.

For labeling reaction using $^{32}$P-γ-labeled ATP use 10 pmol of the labeled ATP, at least 10 pmol substrate and 10 units PNK in its standard buffer as described above. Incubate at 60-70°C for 1-2 hours.

If doing an exchange 5' kinase reaction, increase the incubation time and/or the amount of enzyme used.

### Activity Assay

1x ThermoPhage™ PNK buffer, 25 µg/ml BSA, 2 nmol ATP (mixture of ATP and 32P-γ-ATP) and 1 nmol ssDNA 5' hydroxylated oligonucletide oligomer substrate and 0.1-0.5 unit PNK in 20 µl reaction volume. After incubation at 70°C for 15 minutes, the reactions were terminated by heating it at 95°C for 5 minutes, and the samples captured on DE81 filters and washed twice in 250 mM phosphate buffer. The filter was then dried and counted for radioactivity in a liquid scintillation counter and the incorporation of phosphate determined.

**Product number: Pnk162**
**Lot number:**

## Temperature Stability

ThermoPhage™ Polynucleotide kinase is stable at 60°C but starts to loose activity at extended incubation times above 65°C. Figure 1 shows activity using the standard assay at 50, 60, 65 and 70°C with samples taken at time 0, 30, 60 and 120 min.

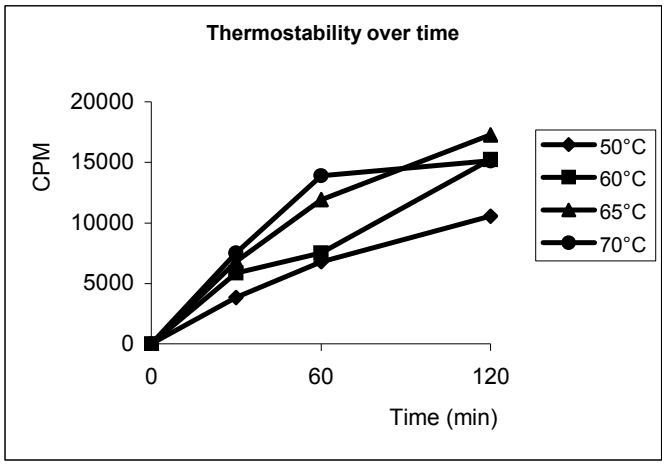


***Fig 1:*** *Thermostability over time at 50-70°C. The enzyme is stable at 50-60°C but starts to loose activity at extended time at 65-70°C.*

## Temperature Optimum

The temperature optimum of the ThermoPhage™ polynucleotide kinase is between 60°C and 70°C. We recommend 70°C for short incubation times (1 hour). For longer incubation times we recommend incubating at 60°C. However, the enzyme shows relatively high activity up to 90°C for shorter incubation times as shown in Figure 2.

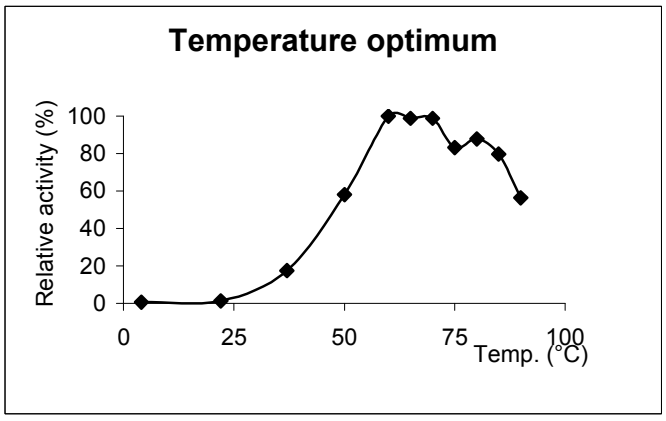


***Fig 2:*** *Activity of ThermoPhage™ Polynucleotide kinase incubated at given temperature for 1 hour under standard assay conditions.*

## 5' Kinase activity

ThermoPhage™ Polynucleotide kinase was assayed with 10 µM ATP (mixture of 32P-γ- and unlabeled ATP) and 20 µM dA20 or rA20 oligomers under standard conditions and 5% PEG6000. Reaction time was 30 min at 70°C in 10 µl reaction volumes. Reactions were terminated by adding stop solution (95% formamide 20 mM EDTA, 0.05% bromophenol blue and 0.05% Xylene Cyanol FF). Samples were run on 20% UREA-PAGE gels, dried and autoradiographed.

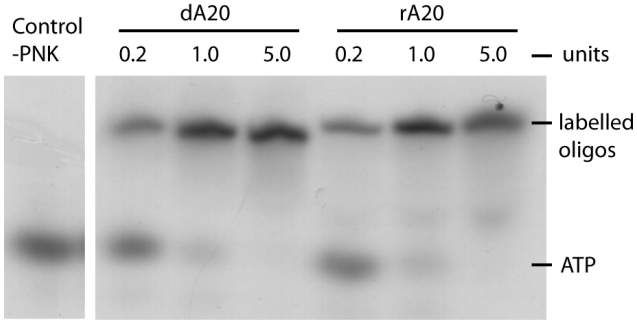


***Fig 3:*** *Phosphorylation of ssDNA (dA20) and RNA (rA20) under standard condition. The PAGE gel shows complete depletion of ATP in the labeling reation using 5 units ThermoPhage™ PNK enzyme.*

## 3' Phosphohydrolase activity

ThermoPhage™ PNK has optimal 3' phosphohydrolase activity at pH 5-7 and mainly in the presence of $Mn^{2+}$ as seen in figure 4. The main substrate is 2'-3' cAMP as seen in figure 5. The 3' hydrolase activity should not interfere with the 5' kinase reaction of the ThermoPhage™ PNK.

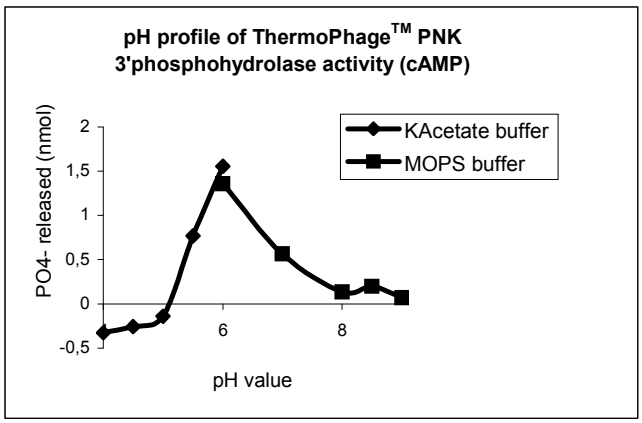


***Fig 4:*** *pH profile of ThermoPhage™ PNK 3' phosphohydrolase activity on 2'-3' cAMP.*

**Product number: Pnk162**
**Lot number:**

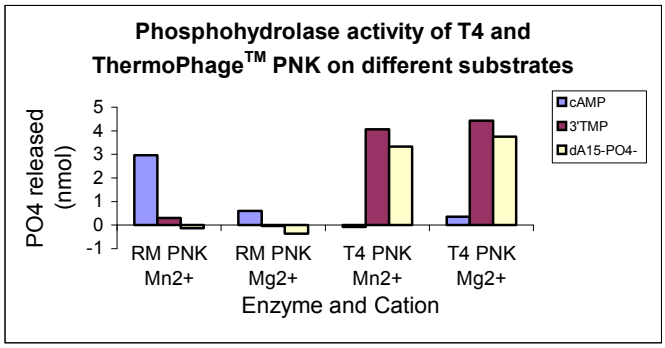


***Fig 5:*** *Comparison of 3' phosphohydrolase activity of T4 PNK and ThermoPhage™ PNK on 100 μM 2'-3'cAMP, 3'TMP and dA15-PO4- substrates.*

## Quality Control

Each lot of ThermoPhage™ Polynucleotide kinase is assayed for activity and for contaminating activities as stated below.

### Absence of DNA endonuclease

- 0,25 µg supercoiled pBR322 DNA is incubated with increasing amounts of ThermoPhage™ PNK in 25 µl reactions at 37°C and 64°C for 4 h. 100 U of Thermo-PhageTM PNK show no relaxation of the supercoiled structure of pBR322 DNA.

- 0,25 µg of λ-DNA Eco RI/HindIII fragments is incubated with ThermoPhage™ PNK in 25 µl reactions at 37°C and 64°C for 4 h. 100 U of ThermoPhage™ PNK show no alteration of the banding pattern.

### Absence of exonuclease

Increasing amounts of ThermoPhage™ PNK are incubated in 50 µl test buffer containing [3H]-labelled DNA at 37°C and 64°C for 4 h. The amount of enzyme, which shows no exonuclease activity is at least 200 units.

### Absence of Rnases

RNaseAlert™ Lab Test Kit (cat no. 1964) from Ambion was used to detect RNase activity according to the manufacturer protocol. No RNase activity was detected after 1 hour incubation of 100 units of ThermoPhage™ PNK.

## References


1. Sambrook, J., Fritsch, E. F., and T., M. (1989) Mo-lecular cloning, a laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY.
2. Hjörleifsdottir, S. et al. (2002) US Patent No. 6,492,161
3. Wu, D. Y. & Wallace, R. B. (1989) Genomics 4, 560-569.
4. Barringer, K., et al. (1990) Gene 89, 117-122.
5. Richardson, C. C. (1965) Proc Natl Acad Sci U S A 54, 158-165.


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- HARMFUL ENZYME-PROTEIN
- Enzymes may cause sensitization by inhalation

## Caution:

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- The safety and efficacy of this product in diagnostic or other clinical use has not been established

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