# T4 Polynucleotide Kinase

**Catalogue number:** MB00801, 500 U

## Description

T4 Polynucleotide Kinase catalyses the transfer of the terminal phosphate of ATP to 5'-hydroxyl termini of polynucleotides such as DNA and RNA, oligonucleotides and 3'-mononucleotides. In the presence of ADP, it will also catalyse the exchange of 5'-terminal phosphate groups and ATP. This enzyme also possesses a 3'-phosphatase and 2', 3' cyclic phosphodiesterase activity.

## Storage

50 mM Tris-HCl, pH 7.6, 1.0 mM DTT, 0.1 mM EDTA, 50% (v/v) glycerol. Store at -20 °C.

**Concentration:** 30 U/µL

## Unit definition

One enzyme unit incorporates 1 nmol of radiolabeled ATP into DNA substrate in 30 min at 37 °C under standard assay conditions.

**Reaction buffer (10×):** 0.5 M Tris-HCl, pH 7.6, 100 mM MgCl2, 100 mM 2-mercaptoethanol. Supplied with dilution buffer: 50 mM Tris-HCl, pH 8.0.

## Related products:

| Product name | Cat. No. |
|---|---|
| Alkaline Phosphatase | MB018 |
| Klenow Fragment of DNA polymerase I | MB009 |
| Speedy Ligase | MB130 |
| NZYGelpure | MB011 |

## 5' – End labelling protocol

1. This protocol is suitable for 10 pmol of oligonucleotide. Combine the following:

| | |
|---|---|
| Oligonucleotide (10 pmol) | __µL |
| [γ-$^{32}$P]ATP* | __µL |
| 10× Reaction buffer | 5 µL |
| Water | __µL |
| T4 Polynucleotide Kinase (diluted) | 2-5 units |
| Final volume | 50 µL |

2. Mix and incubate at 37 °C for 30 min.

3. Terminate the reaction by heating at 65 °C for 10 minutes. Proceed with the separation of 5´-end-labeled oligonucleotide from precursor ATP by thin-layer or column chromatography according to standard methods.

* The number of pmol of [γ-$^{32}$P]ATP should be at least 2 times the number of pmol of 5´-ends.

**Notes:** Dilute the T4 Polynucleotide Kinase using 50 mM Tris-HCl, pH 8.0 (Dilution buffer). Adding concentrated enzyme can result in lower levels of incorporation. Do not store the enzyme in the diluted form. The labelling efficiency is influenced by the terminal 5´ nucleotide. An oligonucleotide with G at the 5´-end labels about 6 fold higher than a 5'C and about 1.5 fold higher than a 5´T or 5´A.

Revised 12/12

## Certificate of Analysis

| Test | Result |
|---|---|
| Enzyme purity | Pass |
| Nucleases assays | Pass |
| Functional assay | Pass |

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