T4 Polynucleotide Kinase

Catalogue number: MB00801, 500 U

Description

T4 Polynucleotide Kinase (T4 PNK) catalyses the transfer of the terminal phosphate of ATP to 5'-hydroxyl termini of polynucleotides such as DNA and RNA, oligonucleotides and 3'-mononucleotides. This enzyme also possesses a 3'-phosphatase and 2', 3' cyclic phosphodiesterase activity. The enzyme can be used for the following applications: 5´-phosphorylation of oligonucleotides, PCR products, other DNA or RNA to allow subsequent ligation; end-labelling DNA or RNA for use as probes, for DNA sequencing or for DNA-protein foot printing; removal of 3' phosphoryl groups.

Storage temperature

T4 Polynucleotide Kinase should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

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

Enzyme concentration: 30 U/µL

Inactivation

T4 Polynucleotide Kinase is heat inactivated at 65 °C for 10 min.

System components and Reaction conditions

T4 Polynucleotide Kinase is provided with a dedicated highly optimized NZYTech reaction buffer, as well as a dilution buffer to optimize levels of incorporation. See section “Important notes” below for further information. The optimum temperature for activity is 37 °C.

End labelling protocol (radioactive 5´-end labelling)

This protocol is suitable for 10 pmol of dephosphorylated DNA or oligonucleotide.

1. Prepare the following 50 µL reaction:

Oligonucleotide (10 pmol)	x µL
[γ-^{32}P]ATP*	y µL
10× Reaction buffer for T4 PNK	5 µL
Nuclease-free H_2O	Up to 50 µL
T4 Polynucleotide Kinase (diluted)	2-5 units
Final volume	50 µL

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

2. Mix gently and pulse.

3. Incubate at 37 °C for 30 min.

4. Stop 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.

DNA phosphorylation protocol

The following standard protocol serves as a general guideline for a non-radioactive kinase reaction. Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Linear dsDNA or oligonucleotide*	x µL
ATP (10 mM) (not provided)	5 µL
10× Reaction buffer for T4 PNK	5 µL
Nuclease-free H_2O	Up to 50 µL
T4 Polynucleotide Kinase (diluted)	10 units
Final volume	50 µL

** up to 300 pmol of 5-end.*

2. Mix gently and pulse.

3. Incubate at 37 °C for 30 min.

4. Stop the reaction by heating at 65 °C for 10 minutes.

Important notes

- Dilute the T4 Polynucleotide Kinase using the Dilution buffer provided. Adding concentrated enzyme can result in lower levels of incorporation. Do not store the enzyme in the diluted form.
- 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.

Quality control assays

Purity

T4 Polynucleotide Kinase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe (NZYTech, Cat. No. MB152) staining.

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 10 U of T4 Polynucleotide Kinase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of T4 Polynucleotide Kinase for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

T4 polynucleotide Kinase is tested for activity in a reaction to phosphorylate an insert DNA required for ligation with a non-phosphorylated vector. The efficiency of Kinase´s reaction is evaluated by the number of bacterial colonies transformed with the cloning product.

Related products

Product name	Cat. No.
Water for Molecular Biology	MB11101
Alkaline Phosphatase	MB018
Speedy Ligase	MB130
NZYGelpure	MB011

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases contamination	Pass
Functional assay	Pass

Approved by:

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