MB008_IFU_EN_V2601

T4 Polynucleotide Kinase

Catalogue number	Presentation
MB00802	20 x 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.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components at -80 °C to -15 °C. These meticulous storage procedures ensure that T4 Polynucleotide Kinase delivers consistent and reliable results across its lifespan and usage. All components are formulated to be ready to use. The kit will remain stable till the expiry date if stored as specified.

Components

COMPONENT	500 U	
	TUBES	VOLUME
T4 Polynucleotide Kinase	1	20 µL
10x Reaction buffer for T4 Polynucleotide Kinase	1	1 mL
Dilution Buffer for T4 Polynucleotide Kinase	1	1 mL

Specifications

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.

Inhibition & Inactivation: T4 Polynucleotide Kinase is heat inactivated at 65 °C for 10 min.

Standard Protocol

System components

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:

	1 REACTION VOLUME
Oligonucleotide (10 pmol)	x µL
[γ-^{32}P]ATP*	y µL
10× Reaction buffer for T4 PNK	5 µL
T4 Polynucleotide Kinase (diluted 1:10)	2-5 units
Nuclease-free water	up to 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:

	1 REACTION VOLUME
Linear dsDNA or oligonucleotide*	x µL
ATP (10 mM) (not provided)	5 µL
10× Reaction buffer for T4 PNK	5 µL
T4 Polynucleotide Kinase (diluted 1:10)	3.2 µL (10 units)
Nuclease-free water	up to 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

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 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.

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