T4 Polynucleotide Kinase

Cat. No. : RK20524

Product Components

Components	Component number	Concentration	Size-1 250 U	Size -2 2,500 U
T4 Polynucleotide Kinase	RM20514	10,000 U/mL	25 μL	250 μL
10X T4 PNK Reaction Buffer	RM20122	10X	1.25 mL	1.25 mL

Product Description

T4 Polynucleotide Kinase (T4 PNK) catalyzes the transfer and exchange of Pi from the γ position of ATP to the 5´-hydroxyl terminus of polynucleotides (double-and single-stranded DNA and RNA) and nucleoside 3´-monophosphates. T4 Polynucleotide Kinase also catalyzes the removal of 3´-phosphoryl groups from 3´-phosphoryl polynucleotides, deoxynucleoside 3´-monophosphates and deoxynucleoside 3´-diphosphates.

T4 PNK is applicable to end-labeling DNA or RNA for probes and DNA sequencing, addition of 5´-phosphates to oligonucleotides to allow subsequent ligation and removal of 3´-phosphoryl groups.

Product Source

An *E.coli* strain that carries the cloned T4 Polynucleotide Kinase gene.

Unit Definition

One unit of enzyme catalyzes the phosphorylation of 20 pmol of fluorescently labeled oligo in 30 min at 37˚C.

Storage Buffer

10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.1 μM ATP pH7.4 @ 25℃

Storage Temperature

-20℃.

Reaction Conditions

1X T4 PNK Reaction Buffer, Incubate at 37℃

1X T4 PNK Reaction Buffer

70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, pH7.6 @ 25℃

Heat Inactivation

65℃ for 20 min

Instructions

Radioactive Labeling with T4 PNK or T4 PNK (3´ phosphatase minus)

1. Set-up the following reaction in a microcentrifuge tube on ice:

Components	Amount
ddH_2O	Up to 50 μL
10X T4 PNK Reaction Buffer	5 μL
DNA	Up to 50 pmol of 5' termini
[γ-^{32}P] ATP (10 mM)	0.25 μL (50 pM)
T4 PNK	2 μL (20 U)

2. Incubate at 37℃ for 30 minutes.

3. Heat inactivate by incubating at 65°C for 20 minutes.

Note: [^{33}P] ATP may be substituted for [^{32}P] ATP.

Non-radioactive Phosphorylation with T4 PNK or T4 PNK (3′ phosphatase minus)

1. Set-up the following reaction in a microcentrifuge tube on ice:

Components	Amount
ddH_2O	Up to 50 µL
10X T4 PNK Reaction Buffer	5 µL
DNA	Up to 300 pmol of 5′ termini
ATP (10 mM)	5 µL
T4 PNK	1 µL (10 U)

2. Incubate at 37°C for 30 minutes.

3. Heat inactivate by incubating at 65°C for 20 minutes.

Note: ATP is not supplied; 1X T4 DNA Ligase Buffer contains 1 mM ATP and can be substituted in non-radioactive phosphorylations (T4 Polynucleotide Kinase exhibits 100% activity in this buffer).

End-labeling Protocol

5′ overhangs can be end-labeled radioactively with T4 Polynucleotide Kinase (PNK), or filled in using the Klenow fragment with labeled dNTPs:

Components	Amount
ddH_2O	Up to 20 µL
10X T4 PNK Reaction Buffer	2 µL
DNA Ladder	1 or 2 µL (1 µg)
[γ -^{32}P] ATP ((3,000 Ci/mM, 5 mCi/mL)	1 µL
T4 PNK	1 µL (10 U)

1. Incubate at 37°C for 30 minutes.

2. Run the samples for 50 to 60 minutes at 100V in TBE buffer in a 4-20% acrylamide gel (10 cm x 10 cm). A 20 minutes exposure gives very readable signals. The signal strength is about twice that signal when ADP is added to 100 µM.

Notes

1. Gaps can be phosphorylated with elevated levels of ATP. Nicks are not phosphorylated efficiently. CTP, GTP, TTP, UTP, dATP or dTTP can be substituted for ATP as a phosphate donor.

2. Dephosphorylation prior to end-labeling can be avoided by utilizing the exchange reaction, although this results in lower specific activity labeling.

3. Sufficient incorporation levels can be attained using the supplied buffer supplemented with 100 µM ADP in the final reaction.