T4 Polynucleotide Kinase

CATALOG NO.: M1216-100

AMOUNT: 1000 U (100 µl)

PRODUCT SOURCE: Recombinant *E. coli*

FORM: Liquid. Enzyme supplied with 10X Reaction Buffer

COMPONENTS:

Components Name	Volume	Part No.
Poly(A) Polynucleotide Kinase (10 U/µl)	100 µl	M1216-100-1
10X T4 Polynucleotide Kinase Reaction Buffer	500 µl	M1216-100-2

DESCRIPTION: T4 Polynucleotide Kinase catalyzes the transfer of the γ-phosphate from ATP to the 5´-hydroxyl terminus of double and single-stranded RNA and DNA, oligonucleotides or nucleoside 3´-monophosphates. The enzyme is also capable of catalyzing the removal of 3´-phosphoryl groups from 3´-phosphoryl polynucleotides, deoxynucleoside 3´-monophosphates and deoxynucleoside 3´-diphosphates.

APPLICATIONS:

1. Labelling 5'-termini of DNA or RNA to be used as:
   a. primers for DNA sequencing
   b. primers for PCR
   c. probes for hybridization
   d. probes for transcript mapping
   e. markers for gel electrophoresis
2. Addition of 5'-phosphates to oligonucleotides, PCR products, and DNA or RNA prior to ligation
3. Removal of 3´-phosphoryl groups

STORAGE CONDITIONS: Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

ENZYME STORAGE BUFFER: 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 µM ATP, and 50% (v/v) Glycerol.

ENZYME UNIT DEFINITION: One unit is defined as the amount of T4 Polynucleotide Kinase that catalyzes the incorporation of 1 nmol γ-phosphate from ATP to the 5'-hydroxyl termini of micrococcal nuclease-treated DNA in 30 minutes at 37°C in 1X T4 Polynucleotide Kinase Reaction Buffer.

10X T4 POLYNUCLEOTIDE KINASE REACTION BUFFER: 700 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, pH 7.6

HEAT INACTIVATION: 65°C for 20 minutes

PROTOCOL:

Forward Labelling Reaction:

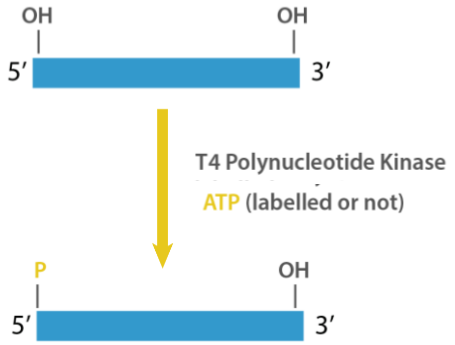
The forward reaction will add a phosphate group to nucleic acids with 5'-hydroxyl groups. Labelled or unlabelled ATP not included in supplied 10X buffer.

1. Add the following components to a sterile tube sitting on ice in the following order:

Components	Volume	Final Concentration
Dephosphorylated DNA fragments	Variable	5 pmol
10X T4 Polynucleotide Kinase Reaction Buffer	2.5 µl	1X
T4 Polynucleotide Kinase	1 µl	10 U
[γ-^{32}P] ATP (10 µCi/µl, 3000 Ci/mmol) *	2.5 µl	0.33 µM
Nuclease-free H_2O	upto 25 µl	-

**To phosphorylate substrate with unlabeled phosphate, add cold ATP to a final concentration of 1 mM (not supplied).*

2. 2. Mix gently and collect all components by a brief centrifugation.
3. Incubate the reaction at 37°C for 10 minutes.
4. Stop the reaction by adding EDTA to 5 mM or by heat inactivating for 10 minutes at 65 °C.



RELATED PRODUCTS:

- Advance™ DNA Polymerase (Cat# M1151-250, -1000)
- Blood Advance™ DNA Polymerase (Cat# M1153-100, -400)
- Breeze™ DNA Polymerase (Cat# M1148-250, -1000)
- Distant™ DNA Polymerase (Cat# M1150-250, -1000)
- Fire Start™ DNA Polymerase (Cat# M1149-250, -1000)
- Outstretched™ DNA Polymerase (Cat# M1152-250, -1000)
- PFU DNA Polymerase (Cat# 9003-500, -2500)
- Ready™ DNA Polymerase (Cat# M1146-1000, -5000, -10000)
- Robust Ready™ DNA Polymerase (Cat# M1147-250, -1000)
- Taq DNA Polymerase (Cat# 9001-500, -2500)

FOR RESEARCH USE ONLY! Not to be used on humans.

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