

T4 Polynucleotide Kinase Technical Specifications



Catalog No. 30061-1 1,000 Units (1 x 100µl)
 Catalog No. 30061-3 5,000 Units (5 x Cat. No. 30061-1).
 Includes 10X Kinase Buffers with and without ATP
 (1ml each per 1,000 Units)
Store at -20°C.
 For *In Vitro* Research Use Only.
 Not for Drug or Diagnostic use. Not for use in humans or animals.

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Product Description	T4 Polynucleotide Kinase, 10,000 Units/ml.
Storage Buffer	50% glycerol, 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 0.1mM EDTA, 1 mM DTT.
Stability	T4 Polynucleotide Kinase, is stable for one year from the date received if stored at -20°C.
Recommended Reaction Conditions	5 U T4 Polynucleotide Kinase; 1X reaction Buffer containing 20 mM Tris-HCl (pH8.5), 2 mM MgCl ₂ , 5 mM DTT and 0.5 mM ATP(See Protocol).
Activity Determination	One unit is defined as the amount of enzyme needed to catalyze the transfer of 1nanomole of phosphate to the 5'-OH end of a polynucleotide from [γ- ³² P]ATP in 30 minutes at 37°C.
Absence of Endonuclease or Nicking Activity	Incubation of 5 units of T4 Polynucleotide Kinase, with 1 µg of supercoiled pBR322 DNA for 16 hours at 37°C resulted in no detectable conversion to relaxed or linear forms by agarose gel electrophoresis.
Absence of Exonuclease Activity	Incubation of 5 units of T4 Polynucleotide Kinase, with 1 µg of HindIII cut lambda DNA for 16 hours at 37°C resulted in no detectable reduction in molecular weight by agarose gel electrophoresis.
Purity	>99% pure by SDS PAGE. No detectable DNA contamination.

Applications

- End-labeling DNA or RNA for probes and DNA sequencing (1).
- Addition of 5'-phosphates to oligonucleotides to allow subsequent ligation (2).

Additional Reagents: Supplied with 10X Kinase Buffer with ATP and 10X Kinase Buffer without ATP.

Heat Inactivation: 70°C for 15 min.

References

- 1) Sambrook, J. and Russell, W. (2001) Molecular Cloning: A Laboratory Manual, (3rd ed.), 9.55-9.56, 9.68-9.69.
- 2) Sambrook, J. and Russell, W. (2001) Molecular Cloning: A Laboratory Manual, (3rd ed.), 13.16-13.17.

Please see reverse side for reaction conditions and Warranty information.

Protocol :

T4 Polynucleotide Kinase requires ATP for its activity. The enzyme is supplied with two buffers: 10X Kinase Buffer without ATP for radioactive labeling and 10X Kinase Buffer with ATP for non-radioactive phosphorylation.

For radioactive labeling, use 1-50 pmol of 5' termini in 50 µl reaction containing 10X Kinase Buffer without ATP, 50 pmol of [γ -³²P]ATP and 20 U of T4 Polynucleotide Kinase for 30 minutes at 37°C.

For non-radioactive phosphorylation, use 300 pmol of 5' termini in 50 µl reaction containing 10X Kinase Buffer with ATP and 20 U of T4 Polynucleotide Kinase for 30 minutes at 37°C.

For non-radioactive phosphorylation to be followed by a ligation, the reaction can be performed directly in T4 DNA Ligase Buffer.

Warranty

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