T4 Polynucleotide Kinase

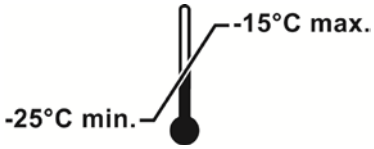
FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE

Lucigen® Simplifying Genomics

Technical Specifications

Catalog No. 30061-1 1,000 Units (1 x 100 µL)
Catalog No. 30061-3 5,000 Units (5 x Cat. No. 30061-1).
Includes 10X Kinase Buffers with and without ATP
(1 mL each per 1,000 Units)

Store at –20ºC.



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, 0.1 µM ATP.
Stability	T4 Polynucleotide Kinase, is stable for one year from the date received if stored at –20ºC.
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^{O}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 37ºC 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 37ºC 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: 70ºC 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.

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^{O}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^{O}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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