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DATA QUALITY SHEET

T4 Polynucleotide Kinase

Description: Catalyses transfer and exchange of Pi from γ position of ATP to 5'-hydroxyl terminus of polynucleotides (double and single-stranded DNA and RNA) and nucleoside 3'-monophosphates. Polynucleotide kinase also catalyses the removal of 3'-phosphoryl groups from 3'-phosphoryl polynucleotides and deoxynucleoside 3'-diphosphates (1). Purified from *E. coli* strain carrying T4 polynucleotide kinase overproducing plasmid.

Unit definition: 1 unit of enzyme transfers 1 nanomole of γ -phosphate from ATP to 5'-OH DNA in 30 min at 37°C.

Activity assay: 100 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 5 mM DTT, 0.5 mM 5'-OH-DNA, 66 mM [γ -³²P] ATP (5 x 10⁶ cpm/ mmole, 0.26 mM hydroxyl-terminated calf thymus DNA (1).

Storage conditions: -200 C in 50 mM KCl, 10 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM DTT, 0.1 mM ATP and 50% glycerol.

Quality control: Endo-, exodeoxyribonucleases, ribonucleases, phosphatase free.

Applications:

1. DNA or RNA 5'-end labelling (2).
2. Incorporation of phosphates in 5'-hydroxyle of oligonucleotides to allow subsequent ligation.
3. Removal of 3'-phosphoryl group.