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## New England Biolabs Certificate of Analysis

Product Name: T4 Polynucleotide Kinase

Catalog Number: M0201L
Concentration: 10,000 U/ml

Unit Definition: One Richardson unit is defined as the amount of enzyme catalyzing

the incorporation of 1 nmol of acid insoluble [33P] in 30 minutes at

37°C.

Packaging Lot Number: 10203708
Expiration Date: 03/2025
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.1 µM ATP ,

50 % Glycerol, (pH 7.4 @ 25°C)

Specification Version: PS-M0201S/L v1.0

T4 Polynucleotide Kinase Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0201LVIAL	T4 Polynucleotide Kinase	10181518	Pass	
B0201SVIAL	T4 Polynucleotide Kinase Reaction Buffer	10201093	Pass	

Assay Name/Specification	Lot # 10203708
DNase Activity (Labeled Oligo, 3' extension) A 50 μl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 50 units of T4 Polynucleotide Kinase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
DNase Activity (Labeled Oligo, 5' extension) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5' extension and a minimum of 50 units of T4 Polynucleotide Kinase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
Double Stranded DNase Activity (Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 50 units of T4 Polynucleotide Kinase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass



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Assay Name/Specification	Lot # 10203708
Endonuclease Activity (Nicking) A 50 μl reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 100 units of T4 Polynucleotide Kinase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ ³H] E. coli DNA and a minimum of 100 units of T4 Polynucleotide Kinase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of T4 Polynucleotide Kinase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Protein Purity Assay (SDS-PAGE) T4 Polynucleotide Kinase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of T4 Polynucleotide Kinase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	Pass
Single Stranded DNase Activity (FAM-Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 50 units of T4 Polynucleotide Kinase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 10 units of T4 Polynucleotide Kinase is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.	Pass

This product has been tested and shown to be in compliance with all specifications.



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Mary Lorenzen Production Scientist 23 Mar 2023

NEW ENGLAND

Josh Hersey

Packaging Quality Control Inspector

22 Sep 2023