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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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 [ <sup>33</sup> P] in 30 minutes at 37°C.
Lot Number:	10032258
Expiration Date:	11/2020
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	10020297	Pass	
B0201SVIAL	T4 Polynucleotide Kinase Reaction Buffer	10016553	Pass	

Assay Name/Specification	Lot # 10032258
<b>Double Stranded DNase Activity (Labeled Oligo)</b> 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
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
<b>Endonuclease Activity (Nicking)</b> 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





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Assay Name/Specification	Lot # 10032258
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] 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
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
<b>Non-Specific DNase Activity (16 Hour)</b> 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
<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 $\leq$ 1 E. coli genome.	Pass
<b>RNase Activity (Extended Digestion)</b> 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

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





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