NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*T4 Polynucleotide Kinase (3' phosphatase minus)*
Catalog Number:	*M0236L*
Concentration:	*10,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid insoluble [^{33}P] in 30 minutes at 37°C.*
Packaging Lot Number:	*10064919*
Expiration Date:	*01/2022*
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-M0236S/L v1.0*

T4 Polynucleotide Kinase (3' phosphatase minus) Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0236LVIAL	T4 Polynucleotide Kinase (3' phosphatase minus)	10064917	**Pass**
B0201SVIAL	T4 Polynucleotide Kinase Reaction Buffer	10056315	**Pass**

Assay Name/Specification	**Lot # 10064919**
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 (3' phosphatase minus) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE) T4 Polynucleotide Kinase (3' phosphatase minus) 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 (3' phosphatase minus) is incubated at 37ºC. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
Double Stranded DNase Activity (Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent	**Pass**

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Assay Name/Specification	Lot # 10064919
labeled double-stranded oligonucleotide containing a blunt end and a minimum of 50 units of T4 Polynucleotide Kinase (3' phosphatase minus) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	
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 (3' phosphatase minus) incubated for 4 hours at 37ºC 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 [^{3}H] E. coli DNA and a minimum of 100 units of T4 Polynucleotide Kinase (3' phosphatase minus) incubated for 4 hours at 37ºC 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 (3' phosphatase minus) incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**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 (3' phosphatase minus) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of T4 Polynucleotide Kinase (3' phosphatase minus) 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**
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 (3' phosphatase minus) 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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27 Jan 2020

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04 Feb 2020