NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*T4 Polynucleotide Kinase (3' phosphatase minus)*
Catalog #:	*M0236S/L*
Concentration:	*10,000 units/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.*
Lot #:	*0041802*
Assay Date:	*02/2018*
Expiration Date:	*2/2020*
Storage Temp:	*-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*
Effective Date:	*03 Feb 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0041802**
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**
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**
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 (3' phosphatase minus) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
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 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0041802**
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 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 (3' phosphatase minus) 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 (3' phosphatase minus) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**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**
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 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 (3' phosphatase minus) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**

Authorized by
Derek Robinson
03 Feb 2017

Inspected by
Mary Lorenzen
14 Feb 2018

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices