NEW ENGLAND BioLabs Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*T4 Polynucleotide Kinase*
Catalog #:	*M0201S/L*
Concentration:	*10,000 units/ml*
Unit Definition:	*One Richardson 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 #:	*0951703*
Assay Date:	*03/2017*
Expiration Date:	*3/2019*
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-M0201S/L v1.0*
Effective Date:	*02 Feb 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0951703**
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**
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 [^{3}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**

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Assay Name/Specification (minimum release criteria)	**Lot #0951703**
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**
qPCR DNA Contamination (*E. coli* Genomic) - 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**
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**

Authorized by
Derek Robinson
02 Feb 2017

Inspected by
Mary Lorenzen
01 Mar 2017

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