

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 [³³P] in 30 minutes at 37°C.
Lot #: 0951711
Assay Date: 11/2017
Expiration Date: 11/2019
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 #0951711
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] <i>E. coli</i> 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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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 (<i>E. coli</i> Genomic) - A minimum of 10 units of T4 Polynucleotide Kinase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> 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
07 Nov 2017

