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New England Biolabs Certificate of Analysis

Product Name: T4 Polynucleotide Kinase (3' phosphatase minus)

Catalog Number: M0236S
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 [33P] in 30 minutes at

37°C.

Lot Number: 10043576
Expiration Date: 06/2021
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.1 μ M 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 | |
| M0236SVIAL | T4 Polynucleotide Kinase (3' phosphatase minus) | 10043577 | Pass | |
| B0201SVIAL | T4 Polynucleotide Kinase Reaction Buffer | 10016553 | Pass | |

| Assay Name/Specification | Lot # 10043576 |
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| 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 [³H] 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 | Pass |



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| 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. | |
| 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 nternal 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 |
| DNase Activity (Labeled Oligo, 3' extension) A 50 μl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent abeled 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 abeled 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 abeled 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 87°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 |

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



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30 Nov 2018



Michael Torullo

Michael Tonello Packaging Quality Control Inspector 26 Jun 2019