

## 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 [<sup>33</sup>P] in 30 minutes at 37°C.  
**Lot Number:** 10033429  
**Expiration Date:** 11/2020  
**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)	10024301	Pass
B0201SVIAL	T4 Polynucleotide Kinase Reaction Buffer	10016553	Pass

Assay Name/Specification	Lot # 10033429
<p><b>DNase Activity (Labeled Oligo, 3' extension)</b>            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 &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass
<p><b>DNase Activity (Labeled Oligo, 5' extension)</b>            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 &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass
<p><b>Double Stranded DNase Activity (Labeled Oligo)</b>            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 &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass

Assay Name/Specification	Lot # 10033429
<p><b>Endonuclease Activity (Nicking)</b> 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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>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 &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> T4 Polynucleotide Kinase (3' phosphatase minus) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> 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.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> 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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>
<p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>

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

*Mary K Lorenzen*

Mary Lorenzen  
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02 Nov 2018

*Michael Tonello*

Michael Tonello  
Packaging Quality Control Inspector  
14 Jan 2019