

## New England Biolabs Certificate of Analysis

**Product Name:** T4 Polynucleotide Kinase  
**Catalog Number:** M0201S  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One Richardson 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:** 10033445  
**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-M0201S/L v1.0

T4 Polynucleotide Kinase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0201SVIAL	T4 Polynucleotide Kinase	10020296	Pass
B0201SVIAL	T4 Polynucleotide Kinase Reaction Buffer	10016553	Pass

Assay Name/Specification	Lot # 10033445
<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 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 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 incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass

Assay Name/Specification	Lot # 10033445
<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 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 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 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 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 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 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 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*

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Mary Lorenzen  
Production Scientist  
02 Nov 2018

*Michael Tonello*

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Michael Tonello  
Packaging Quality Control Inspector  
01 Mar 2019