

## 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 [<sup>33</sup>P] in 30 minutes at 37°C.  
**Packaging Lot Number:** 10152086  
**Expiration Date:** 06/2024  
**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

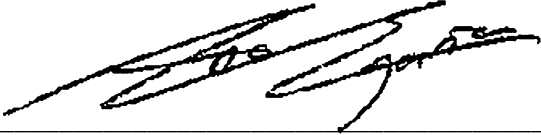
| T4 Polynucleotide Kinase (3' phosphatase minus) Component List |   |            |                      |
|--|---|------------|----------------------|
| NEB Part Number  | Component Description                           | Lot Number | Individual QC Result |
| M0236SVIAL   | T4 Polynucleotide Kinase (3' phosphatase minus) | 10152085   | Pass                 |
| B0201SVIAL   | T4 Polynucleotide Kinase Reaction Buffer        | 10119056   | Pass                 |

| Assay Name/Specification   | Lot # 10152086 |
|--|----------------|
| <p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b><br/>           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>    |
| <p><b>RNase Activity (Extended Digestion)</b><br/>           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>Protein Purity Assay (SDS-PAGE)</b><br/>           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><br/>           A minimum of 10 units of T4 Polynucleotide Kinase (3' phosphatase minus) is screened</p>   | <b>Pass</b>    |

| Assay Name/Specification  | Lot # 10152086 |
|---|----------------|
| <p>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 <math>\leq 1</math> E. coli genome.</p>   |                |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>A 50 <math>\mu</math>l reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 <math>\mu</math>g 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>Exonuclease Activity (Radioactivity Release)</b><br/>A 50 <math>\mu</math>l reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 <math>\mu</math>g 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>Endonuclease Activity (Nicking)</b><br/>A 50 <math>\mu</math>l reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1 <math>\mu</math>g 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>DNase Activity (Labeled Oligo, 3' extension)</b><br/>A 50 <math>\mu</math>l 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> | <b>Pass</b>    |
| <p><b>DNase Activity (Labeled Oligo, 5' extension)</b><br/>A 50 <math>\mu</math>l 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> | <b>Pass</b>    |
| <p><b>Double Stranded DNase Activity (Labeled Oligo)</b><br/>A 50 <math>\mu</math>l 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>  | <b>Pass</b>    |

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

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12 Oct 2022



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12 Oct 2022