240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Product Specification

Product Name: T4 Polynucleotide Kinase

Catalog #: M0201S/L
Concentration: 10,000 units/ml

Unit Definition: One unit of enzyme catalyzes the phosphorylation of 20 pmol of fluorescently labeled oligo in 30 minutes at 37°C.

Shelf Life: 24 month
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 v2.0
Effective Date: 29 May 2024

## Assay Name/Specification (minimum release criteria)

DNase Activity (Labeled Oligo, 3' extension) - A 50  $\mu$ 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 incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

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.

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.

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1  $\mu$ g 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.

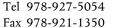
Exonuclease Activity (Radioactivity Release) - A 50  $\mu$ l reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1  $\mu$ g of a mixture of single and double-stranded [  $^3$ H] *E. coli* DNA and a minimum of 100 units of T4 Polynucleotide Kinase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50  $\mu$ l reaction in T4 Polynucleotide Kinase Reaction Buffer containing 1  $\mu$ g 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.









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Assay Name/Specification (minimum release criteria)

**Protein Purity Assay (SDS-PAGE)** - T4 Polynucleotide Kinase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - 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  $\leq 1$  *E. coli* genome.

RNase Activity (Extended Digestion) - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1  $\mu$ l 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.

Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50  $\mu$ l 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.

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Date 29 May 2024

Lauren Brown Quality Approver





