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New England Biolabs Certificate of Analysis

Product Name: Salt-T4® DNA Ligase

Catalog Number: M0467L
Concentration: 400,000 U/ml

Unit Definition: One unit is defined as the amount of enzyme required to give 50%

ligation of 6 μg of Lambda-HindIII DNA in 30 minutes at 25°C in a total reaction volume of 20 μl in 1X T4 DNA Ligase Reaction Buffer

supplemented with 100 mM NaCl.

Packaging Lot Number: 10169459
Expiration Date: 07/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCI, 50 mM KCI, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol,

(pH 7.4 @ 25°C)

Specification Version: PS-M0467S/L v1.0

Salt-T4® DNA Ligase Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0467LVIAL	Salt-T4® DNA Ligase	10162035	Pass	
B5019AVIAL	1 M NaCl	10109974	Pass	
B0535AVIAL	StickTogether™ DNA Ligase Buffer	10130276	Pass	
B0202SVIAL	T4 DNA Ligase Reaction Buffer	10143296	Pass	

Assay Name/Specification	Lot # 10169459
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 400 units of Salt-T4™ DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
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 2000 units of Salt-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
Protein Purity Assay (SDS-PAGE) Salt-T4™ DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie	Pass



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Assay Name/Specification	Lot # 10169459
Blue detection.	
qPCR DNA Contamination (E. coli Genomic) A minimum of 400 units of Salt-T4™ DNA Ligase 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.	Pass
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 Salt-T4™ DNA Ligase 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
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 2000 units of Salt-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
Endonuclease Activity (Nicking) A 50 μl reaction in NEBuffer 1 containing 1 μg of supercoiled PhiX174 DNA and a minimum of 400 units of Salt-T4™ DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Protein Concentration (A280) The concentration of Salt-T4™ DNA Ligase is 0.4 mg/ml +/- 10% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 57,675 and molecular weight of 56,894 daltons for Salt-T4™ DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).	Pass
Single Stranded DNase Activity (FAM-Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 2000 units of Salt-T4™ DNA Ligase 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 labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 2000 units of Salt-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass



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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

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Production Scientist

12 Aug 2021

Michael Tonello

Packaging Quality Control Inspector

24 Oct 2022