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

## New England Biolabs Certificate of Analysis

Product Name:	Salt-T4® DNA Ligase
Catalog Number:	M0467S
Concentration:	400,000 U/ml
Unit Definition:	One unit is defined as the amount of enzyme required to give 50% ligation of 6 $\mu$ g of Lambda-HindIII DNA in 30 minutes at 25°C in a total reaction volume of 20 $\mu$ l in 1X T4 DNA Ligase Reaction Buffer supplemented with 100 mM NaCl.
Packaging Lot Number:	10222605
Expiration Date:	12/2025
Storage Temperature:	-20°C
Storage Conditions:	10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version:	PS-M0467S/L v2.0

Salt-T4® DNA Ligase Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0467SVIAL	Salt-T4V® DNA Ligase	10221176	Pass	
B5019AVIAL	1 M NaCl	10186777	Pass	
B0535AVIAL	StickTogether™ DNA Ligase Buffer	10208824	Pass	
B0202SVIAL	T4 DNA Ligase Reaction Buffer	10213891	Pass	

Assay Name/Specification	Lot # 10222605
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
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
Double Stranded DNase Activity (Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent	Pass





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Assay Name/Specification	Lot # 10222605
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.	
<b>Endonuclease Activity (Nicking)</b> A 50 $\mu$ I reaction in NEBuffer 1 containing 1 $\mu$ g 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
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 $\mu$ I reaction in NEBuffer 1 containing 1 $\mu$ g 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
<b>Protein Purity Assay (SDS-PAGE)</b> Salt-T4® DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>RNase Activity (Extended Digestion)</b> A 10 $\mu$ I reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ I 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
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
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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

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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Alin Dah

Alison Dolan Production Scientist 18 Dec 2023

Michae Tu l

Michael Tonello Packaging Quality Control Inspector 22 Jan 2024

