

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: 10058740
Expiration Date: 11/2021
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 v1.0

Salt-T4™ DNA Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0467LVIAL	Salt-T4™ DNA Ligase	10058612	Pass
B5019AVIAL	1 M NaCl	10052366	Pass
B0535AVIAL	StickTogether™ DNA Ligase Buffer	10053903	Pass
B0202SVIAL	T4 DNA Ligase Reaction Buffer	10041768	Pass

Assay Name/Specification	Lot # 10058740
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
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
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	Pass

Assay Name/Specification	Lot # 10058740
<p>units of Salt-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.</p>	
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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).</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) Salt-T4™ DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass

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



Mary Lorenzen
Production Scientist
09 Sep 2019



Michael Tonello
Packaging Quality Control Inspector
11 Nov 2019