

## 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:** 10186776  
**Expiration Date:** 02/2025  
**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 |
| M0467LVIAL                         | Salt-T4® DNA Ligase              | 10179334   | Pass                 |
| B5019AVIAL                         | 1 M NaCl                         | 10186777   | Pass                 |
| B0535AVIAL                         | StickTogether™ DNA Ligase Buffer | 10164467   | Pass                 |
| B0202SVIAL                         | T4 DNA Ligase Reaction Buffer    | 10153865   | Pass                 |

| Assay Name/Specification  | Lot # 10186776 |
|---|----------------|
| <b>DNase Activity (Labeled Oligo, 3' extension)</b><br>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           |
| <b>DNase Activity (Labeled Oligo, 5' extension)</b><br>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           |
| <b>Double Stranded DNase Activity (Labeled Oligo)</b><br>A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent  | Pass           |

| Assay Name/Specification  | Lot # 10186776 |
|---|----------------|
| <p>labeled double-stranded oligonucleotide containing a blunt end and a minimum of 2000 units of Salt-T4<sup>®</sup> DNA Ligase incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>  |                |
| <p><b>Endonuclease Activity (Nicking)</b><br/>A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 400 units of Salt-T4<sup>®</sup> DNA Ligase 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>Non-Specific DNase Activity (16 Hour)</b><br/>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<sup>®</sup> 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>   | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>Salt-T4<sup>®</sup> DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</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 Salt-T4<sup>®</sup> DNA Ligase 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>Single Stranded DNase Activity (FAM-Labeled Oligo)</b><br/>A 50 µl reaction in CutSmart<sup>®</sup> Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 2000 units of Salt-T4<sup>®</sup> DNA Ligase incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>   | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>A minimum of 400 units of Salt-T4<sup>®</sup> DNA Ligase is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> 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> | <b>Pass</b>    |

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

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10 Feb 2023

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25 Apr 2023