

New England Biolabs Certificate of Analysis

Product Name: *Hi-T4™ DNA Ligase*
Catalog Number: *M2622S*
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.*
Packaging Lot Number: *10267719*
Expiration Date: *04/2026*
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-M2622S/L v3.0*

| Hi-T4™ DNA Ligase Component List | | | |
|----------------------------------|----------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| M2622SVIAL | Hi-T4™ DNA Ligase | 10238812 | Pass |
| B0535AVIAL | StickTogether™ DNA Ligase Buffer | 10256348 | Pass |
| B0202SVIAL | T4 DNA Ligase Reaction Buffer | 10259600 | Pass |

| Assay Name/Specification | Lot # 10267719 |
|---|----------------|
| 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 Hi-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 Hi-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 labeled double-stranded oligonucleotide containing a blunt end and a minimum of 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as | Pass |

| Assay Name/Specification | Lot # 10267719 |
|---|----------------|
| determined by capillary electrophoresis. | |
| <p>Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 400 units of Hi-T4™ DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel 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 Hi-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 Purity Assay (SDS-PAGE) Hi-T4™ DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</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 Hi-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 Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.</p> | Pass |
| <p>qPCR DNA Contamination (E. coli Genomic) A minimum of 400 units of Hi-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 |

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

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20 May 2024



Josh Hersey
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02 Dec 2024