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New England Biolabs
Certificate of Analysis

Product Name:	*Immobilized T4 DNA Ligase*
Catalog Number:	*M0569S*
Packaging Lot Number:	*10098368*
Expiration Date:	*02/2023*
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-M0569S/L v1.0*

Immobilized T4 DNA Ligase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0569SVIAL	Immobilized T4 DNA Ligase	10098365	**Pass**
B0202SVIAL	T4 DNA Ligase Reaction Buffer	10096276	**Pass**

Assay Name/Specification	**Lot # 10098368**
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 Immobilized 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**
qPCR DNA Contamination (E. coli Genomic) A minimum of 10 µg of Immobilized 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**
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 10 µg of Immobilized T4 DNA Ligase incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Functional Testing (Targeted Ligation) A 20 µl reaction in 1X Quick Ligation Buffer containing 20 pmol of a FAM-labeled RNA and an excess of duplex DNA adaptor with 1 µg of Immobilized T4 DNA Ligase incubated for 10 minutes at 25°C results in ≥90% ligation as determined by capillary	**Pass**

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Assay Name/Specification	Lot # 10098368
electrophoresis.	
Functional Testing (Magnetic Beads, Leaching) Immobilized T4 DNA Ligase (100 µl) was incubated in storage buffer for 24 hours at room temperature. The beads were pelleted using a magnetic separation rack and the supernatant boiled in SDS-PAGE buffer for 5 minutes at 100°C. No Immobilized T4 DNA Ligase was detected in the supernatant as determined by an absence of bands visualized using Tris-Glycine gel electrophoresis with Coomassie Blue detection.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 µg of Immobilized 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**
Functional Testing (Bead Concentration) In triplicate reactions, Immobilized T4 DNA Ligase (500 µl) was delivered to tared microcentrifuge tubes. The beads are pelleted using a magnetic separation rack and washed sequentially 3 times with 1 mL of Milli Q water using the magnetic separation rack to pellet the beads and remove the supernatant each time. The microcentrifuge tubes containing the pelleted, desalted beads were incubated at 95°C for 4 hours and weighed to determine the concentration of the beads to be 10.0 ± 0.3 mg/ml.	**Pass**

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

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Production Scientist
08 Feb 2021

Josh Hersey
Packaging Quality Control Inspector
08 Feb 2021