NEW ENGLAND BioLabs® Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Product Specification

Product Name:	*Immobilized T4 DNA Ligase*
Catalog #:	*M0569S/L*
Concentration:	*10 mg/ml*
Shelf Life:	*24 months*
Storage Temp:	*-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*
Effective Date:	*12 Jan 2021*

Assay Name/Specification (minimum release criteria)

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.

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.

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.

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 electrophoresis.

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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

NEW ENGLAND BioLabs® Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Product Specification

Assay Name/Specification (minimum release criteria)
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.
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.

Date 12 Jan 2021

Derek Robinson
Director, Quality Control