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: T4 RNA Ligase 1 (ssRNA Ligase), High Concentration

Catalog #: M0437M

Concentration: 30,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nanomole of fluorescently labeled rA16 into a

phosphatase-resistant form in 30 minutes at 37°C.

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-M0437M v2.0
Effective Date: 23 Oct 2024

## Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in T4 RNA Ligase 1 Reaction Buffer containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase), High Concentration incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 μl reaction in T4 RNA Ligase 1 Reaction Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup>H] *E. coli* DNA and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase), High Concentration incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Protein Purity Assay (SDS-PAGE)** - T4 RNA Ligase 1 (ssRNA Ligase), High Concentration is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (E. coli Genomic) - A minimum of 10 units of T4 RNA Ligase 1 (ssRNA Ligase), High Concentration 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  $\leq 1$  E. coli genome.

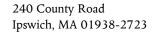
RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of RNA transcript and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase), High Concentration is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using gel electrophoresis.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit <a href="www.neb.com/trademarks">www.neb.com/trademarks</a> for additional information.

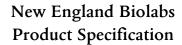








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Haurenbrow

Date 23 Oct 2024

Lauren Brown Quality Approver





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