| Product Information | |
|---|---|
| **T4 RNA Ligase 1** | |
| **Part Number** | L6050L |
| **Concentration** | 20,000 U/mL |
| **Unit Size** | 10,000 U |
| **Storage Temperature** | -25°C to -15°C |
| **Lot Number** | |
| **Reference Number** | |

**Product Description:** T4 RNA Ligase catalyzes the ATP-dependent ligation of single-stranded nucleic acids (RNA or DNA) by joining a 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor through the formation of a 3'→5' phosphodiester bond (1).

| Product Specifications | | | | | | | |
|---|---|---|---|---|---|---|---|
| L6050 | | | | | | | |
| **Assay** | **SDS Purity** | **Specific Activity** | **SS Exonuclease** | **DS Exonuclease** | **DS Endonuclease** | ***E. coli* DNA Contamination** | **Non-specific RNAse** |
| **Units Tested** | **n/a** | **n/a** | **200** | **200** | **200** | **200** | **200** |
| **Specification** | >99% | 16,800 U/mg | <5.0% Released | <1.0% Released | No Conversion | <10 copies | No detectable non-specific RNAse |

**Source of Protein:** Recombinant *E. coli* strain carrying the cloned T4 RNA Ligase gene from bacteriophage T4.

**Unit Definition:** 1 unit is defined as the amount of enzyme required to ligate 50% of 0.4 µg of an equimolar mix of two single stranded 23 base RNA oligonucleotides (one 5'-phosphorylated) in 20 µL 1X T4 RNA Ligase Buffer following a 30 minutes incubation at 37°C.

**Molecular weight:** 43.5 kDa

**Quality Control Analysis:**

**Unit Activity** is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X T4 RNA Ligase reaction buffer and added to 20 µL reactions containing 0.4 µg of an equimolar mix of two single-stranded 23 base RNA oligonucleotides (one 5'-phosphorylated) and 1X T4 RNA Ligase Buffer. Reactions were incubated 30 minutes at 37°C, stopped, and analyzed on a 15% TBE-Urea gel stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen S-11494).

**Protein Concentration ($OD_{280}$)** is determined by $OD_{280}$ absorbance.

**Physical Purity** is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

**Single-Stranded Exonuclease** is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

**Double-Stranded Exonuclease** is determined in a 50 µL reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

**Double-Stranded Endonuclease** is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

**Non-Specific RNAse** contamination is assessed using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.

**Supplied in:**
10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

**Supplied with:**
**10X T4 RNA Ligase Buffer (B6050):** 500 mM Tris-HCl, 100 mM $MgCl_2$, 10 mM ATP, 100 mM DTT (pH 7.8 at 25°C)

**Usage Instructions:** Single-stranded RNA circularization

1. Set up the following reaction mixture in a total volume of 20 µL:

| Components | Final Concentration | Volume |
|---|---|---|
| Nuclease free water | N/A | X µL |
| 10X T4 RNA Ligase Buffer (B6050) | 1X | 2 µL |
| ssRNA with 5'P and 3'OH ends | 200ng - 1µg | X µL |
| RNAse Inhibitor (Y9240) | 20 U | 0.5 µL |
| T4 RNA Ligase 1 (L6050L) | 10 U | 0.5 µL |
| | **Total Volume =** | 20 µL |

2. Incubate at 25°C for 1-2 hours.
3. Reaction can be stopped by adding EDTA to a final concentration of 12.5mM, or clean-up by using a spin column-based method.

**Notes:**
1. For hard to ligate single-stranded substrates, ligation efficiency can be improved by addition of PEG at a final concentration of 5%-10%.
2. For longer single-stranded RNA substrates, overnight incubation at 16°C can improve yield.

**References:**
1. Silber, R. et al. (1972) Proc. Natl. Acad. Sci. USA, 69(10):300-3013.

**Disclaimer:**
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