T4 RNA Ligase 1

Cat. No.	size
E1059-01	1 000 units

Concentration: 10 000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nmole of 5´-[^{32}P]rA$_{16}$ into a phosphatase-resistant form in 30 minutes at 37°C.

Quality Control: All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, single- and double-stranded DNase activities. Greater than 90% as determined by SDS-PAGE.

References:

1. *Uhlenbeck, O.C., Gumport, R.I., T4 RNA ligase,The Enzymes, 15B, 31-60, Academic Press Inc.,1982.*
2. *Middleton, T., et al., Synthesis and purification ofoligoribonucleotides using T4 RNA ligase and reverse-phase chromatography, Anal. Biochem., 144, 110-117, 1985.*
3. *Brennan, C.A., et al., Using T4 RNA ligase with DNA substrates, Meth. Enzymol., 100, 38-52, 1983.*
4. *Tessier, D.C., et al., Ligation of single-stranded oligodeoxyribonucleotides by T4 RNA ligase, Anal. Biochem., 158, 171-178, 1986.*
5. *Heckler, T.G., et al., T4 RNA ligase mediated preparation of novel "chemically misacylated" tRNA Phes, Biochemistry, 23,1468-1473, 1984.*
6. *Edwards, J.B., et al., Oligodeoxyribonucleotide ligation to single-stranded cDNAs: a new tool for cloning 5'-ends of mRNAs and for constructing cDNA libraries by in vitro amplification, Nucleic Acids Res., 19, 5227-5232, 1991.*
7. *Kaluz, S., et al., Enzymatically produced composite primers: an application of T4 RNA ligase-coupled primers to PCR, BioTechniques, 19, 182-186, 1995.*

T4 RNA Ligase 1 catalyzes the ligation of a 5´ phosphoryl-terminated nucleic acid donor to a 3´ hydroxyl-terminated nucleic acid acceptor through the formation of a 3´ → 5´ phosphodiester bond with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Applications:

- RNA 3' -end labeling with Cytidine 3',5' - bis [α-32P] phosphate (1).
- Joining RNA to RNA (2).
- Synthesis of oligoribonucleotides and oligodeoxyribonucleotides (3, 4).
- Specific modifications of tRNAs (5).
- Oligodeoxyribonucleotide ligation to single-stranded cDNAs for 5'-RACE (Rapid Amplification of cDNA Ends) (6).
- Site-specific generation of composite primers for PCR (7).

Source: An *E. coli* strain containing a clone of the T4 RNA ligase gene.

Format: liquid

Storage Buffer: 20 mM HEPES (pH 7.4), 1 mM EDTA, 2 mM DTT, 50% glycerol.

T4 RNA Ligase Reaction Buffer (1x): 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 1 mM DTT.

RNA-RNA ligation exemplary reaction (20 µl):

Component:	Amount:
10 x T4 RNA Ligase Reaction Buffer	2 µl
RNase Inhibitor (E4210)	20 U
ATP	1 mM
DMSO (optional)	10%
ssRNA acceptor with free 3' -OH	20 pmol
RNA donor with free 5' -PO_4 and blocked 3' end	up to 200 pmol
T4 RNA Ligase	10 U
RNase-free Water	to 20 µl

Incubate at 25°C for 2 hours or at 16°C for 16 hours, proceed to RNA clean up for further RNA manipulation steps.

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