T4 RNA Ligase 1 (ssRNA Ligase)

1-800-632-7799
info@neb.com
www.neb.com

M0204S 049121214121

M0204S

RR 37° Yes

1,000 units **10,000 U/ml** **Lot: 0491212**
RECOMBINANT **Store at –20°C** **Exp: 12/14**

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

Source: An *E. coli* strain that carries the T4 RNA Ligase 1 gene

Applications:
- Ligation of ss-RNA and DNA
- Labeling of 3´-termini of RNA with 5´-[^{32}P] pCp (3)
- Inter- and intramolecular joining of RNA and DNA molecules (4,5)
- Synthesis of single-stranded oligodeoxyribonucleotides (6)
- Incorporation of unnatural amino acids into proteins (7)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X T4 RNA Ligase Reaction Buffer, 10 mM ATP and 50% PEG 8000.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer, supplemented with 1 mM ATP. Incubate at 37°C.

1X T4 RNA Ligase Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.5 @ 25°C

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

Unit Assay Conditions: 1X T4 RNA Ligase reaction buffer, supplemented with 1 mM ATP, is mixed with the RNA substrate (10µM of 5´-[$_{32}$P]rA_{16}) and varying amounts of enzyme. Incubation is at 37°C for 15 minutes (8).

Heat Inactivation: 65°C for 15 minutes or boiling for 2 minutes.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 20 units of T4 RNA Ligase 1 with 40 ng of RNA transcript for 2 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

DNA Exonuclease Activity: Incubation of 20 units of T4 RNA Ligase 1 with 1 µg of mixed single and double-stranded sonicated ^{3}H DNA (10^5 cpm/µg) in 50 µl T4 RNA Ligase Reaction Buffer for 4 hours at 37°C released < 0.1% of the activity.

DNA Endonuclease Activity: Incubation of 20 units of T4 RNA Ligase 1 with 1 µg ϕX174 RF I DNA in 50 µl T4 RNA Ligase Reaction Buffer for 4 hours at 37°C resulted in no detectable degradation of DNA as determined by agarose gel electrophoresis.

Notes on Use: Addition of DMSO to 10% (v/v) is required for pCp ligation (3).

(see other side)

CERTIFICATE OF ANALYSIS

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References:

1. England, T., Gumport, R. and Uhlenbeck, O. (1977) *Proc. Natl. Acad. Sci. USA* 74, 4839–4842.
2. Rand, K.N. and Gait, M.J. (1984) *EMBO J.* 3, 397–402.
3. England, T. and Uhlenbeck, O. (1978) *Nature* 275, 560–562.
4. Romaniuk, P. and Uhlenbeck, O. (1983). In R. Wu, L. Grossman and K. Moldave (Eds.), *Methods in Enzymology* Vol. 100, (pp.52–56). New York: Academic Press.
5. Moore, M.J. and Sharp, P.A. (1992) *Science* 256, 992–997.
6. Tessier, D.C., Brousseau, R. and Vernet, T. (1986) *Anal. Biochem.* 158, 171–178.
7. Noren, C.J. et al. (1989) *Science* 244, 182–188.
8. Silber, R., Malathi, B.G. and Hurwitz, J. (1972). *Proc. Natl. Acad. Sci. USA* 69, 3009–3013.

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