T4 RNA Ligase 2, truncated KQ

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

M0373S 002170319031

M0373S

RR 25° Yes

2,000 units	**200,000 U/ml**	**Lot: 0021703**
RECOMBINANT	**Store at –20°C**	**Exp: 3/19**

Description: T4 RNA Ligase 2, truncated KQ (T4 Rnl2tr R55K, K227Q) specifically ligates the pre-adenylated 5´ end of DNA or RNA to the 3´ OH end of RNA. The enzyme does not use ATP for ligation but requires pre-adenylated linkers.

T4 Rnl2tr R55K, K227Q is a double-point mutant of T4 RNA Ligase 2, truncated (NEB #M0242). Mutation of K227 in T4 RNA Ligase 2 reduces enzyme lysyl adenylation (1). K227Q reduces the formation of undesired ligation products (concatemers and circles) by T4 Rnl2tr (2,3), by reducing the trace activity of T4 Rnl2tr in transfer of adenylyl groups from linkers to the 5´-phosphates of input RNAs (3). Mutation of R55K in T4 Rnl2tr K227Q increases the ligation activity of the enzyme to levels similar to T4 Rnl2tr (3)

The exclusion of ATP, use of pre-adenylated linkers, and the reduced enzyme lysyl adenylation activity provide the lowest possible background in ligation reactions. This enzyme has been used for optimized linker ligation for high-throughput sequencing library construction of small RNA (4).

Source: T4 Rnl2tr R55K, K227Q is expressed as an MBP fusion from a plasmid in *E. coli* which encodes the first 249 amino acids of the full length T4 RNA Ligase 2 with an arginine to lysine and a lysine to glutamine mutations at positions 55 and 227, respectively.

Applications:

- Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´-end
- Join a single stranded adenylated primer to small RNAs for cDNA library creation
- Join a single stranded adenylated primer to RNA for strand-specific cDNA library construction.

Supplied in: 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT and 50% glycerol.

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

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Incubate at 25°C.

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

Note: In our tests, this ligase can be used interchangeably with T4 Rnl2tr (NEB #M0242). Ligation activity is stimulated by adding PEG (See FAQ).

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA [universal miRNA cloning linker (NEB #S1315)] in a total reaction volume of 20 µl in 1 hour at 25°C.

5´-FAM- rArGrUrCrGrUrArGrCrCrUrUrUrArUrCrCrGrArGrArUrUrCrArGrCrArArUrA-3´

5´-rAppCTGTAGGCACCATCAAT–NH_2-3´

Unit Assay Conditions: 1X T4 RNA Ligase Reaction Buffer supplemented to 10% (w/v) PEG MW 8000, 20 pmol of 5´-FAM labeled RNA, and 40 pmol preadenylated DNA linker. After incubation at 25°C for 1 hour, the ligated product is detected on a 15% denaturing polyacrylamide gel.

Molecular Weight: 71,378.96 daltons.

Specific Activity: 200,000 U/mg

Molarity: 14 µM

Heat Inactivation: 65°C for 20 minutes.

Quality Control Assays

RNase Assay: A 10 µl reaction in T4 RNA Ligase Reaction Buffer containing 40 ng of labeled RNA and 200 units of T4 RNA Ligase 2, truncated KQ is incubated at 25°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by polyacrylamide electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

Exonuclease Activity: Incubation of a 50 µl reaction containing 200 units of T4 RNA Ligase 2, truncated KQ with 1 µg of a mixture of single and double-stranded ^{3}H *E. coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 200 units of T4 RNA Ligase 2, truncated KQ with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of 200 units of T4 RNA Ligase2, truncated KQ with 2.5 µmol *p*-nitrophenyl phosphate (PNPP) in 50 µl T4 RNA Ligase Reaction Buffer for 3 hours at 37°C released less than 0.05 µmol inorganic phosphate.

References:

1. Yin, S., Ho, C.K. and Shuman S. (2003) *J. Biol. Chem.* 278, 17601–17608.
2. Hafner, M., et al. (2008) *Methods* 44, 3–12.
3. Viollet, S., et al. (2011) *BMC Biotechnol.* 11:72.
4. Zhuang, F., et al. (2012) *Nucleic Acids Res.* 40(7) e54.

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