T4 RNA Ligase 2

Product Description (Product Number #RNL2001)

T4 RNA Ligase 2 catalyzes the ATP-dependent ligation of a 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor by forming a 3'-5' phosphodiester bond. The preferred substrate is double stranded RNA but the ligase is able to seal nicks in DNA-RNA hybrids as well, given that the 3' OH is provided by RNA.

The enzyme is supplied at a concentration of ~ U/µl in Dilution Buffer.
Store at -80 °C. It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.
For *in vitro* laboratory research use only.

Dilution Buffer

50 mM Tris.HCl (pH 7.5)
100 mM NaCl
0.1 mM EDTA
2 mM DTT
50 % (w/v) glycerol

Assay Buffer (supplied as 2x stock)

50 mM Tris.HCl (pH 7.5)
10 mM $MgCl_2$
2 mM DTT

Unit Definiton

One cohesive end ligation unit (CELU) is the amount of enzyme which gives 50% ligation.

Ligation Assay

ds RNA-DNA substrate (RNA in red, DNA in black)

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5'-Flu-GGC CAG UG-AAT TCG AGC TCG-3'
   3'- CCG GTC AC TTA AGC TCG AGC-5'
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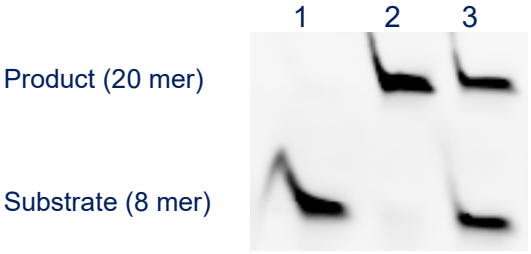
10 pmols of a double stranded 20-mer with a nick in one strand, 5' end-labelled on the nicked strand, is incubated with ligase and 1x Assay Buffer supplemented with 1 mM ATP, at 37°C for 30 minutes, run on a 15 % (w/v) acrylamide/8M urea gel and visualised using a Fujifilm Fla-7000 Image reader.
1= no ligase ; 2, 3 enzyme dilutions: 2= 1/1000 dilution ; 3= 1/5000 dilution.



Quality Control

Purity: The enzyme is purified to >95% purity as judged by SDS-polyacrylamide gel electrophoresis.

Endonuclease assay: 0.25 µg supercoiled pBR322 incubated with ~10 U of RNA Ligase 1 for 4 hours at 37°C in the presence of 1 mM ATP shows no detectable conversion of superhelical DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.

Exonuclease assay: ~10 U of RNA Ligase 1 was incubated with an internally labelled single stranded DNA substrate containing 20 bases (0.6 µM) for 4 hours at 37°C and no detectable degradation was seen when visualised using a Fujifilm Fla-7000 Image reader.

References

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