T4 RNA Ligase 2

Product Description (Product Number #RNL2001)

T4 RNA Ligase 2 catalyses 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 provided that the 3' OH is provided by RNA.

The enzyme is supplied at a concentration of ~10 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

10 mM Tris.HCl (pH 7.5 @ 25°C)
50 mM KCl
35 mM Ammonium sulphate
0.1 mM EDTA
0.1 mM DTT
50 % (w/v) glycerol

Assay Buffer (supplied as 10x stock)

50 mM Tris.HCl (pH 7.5 @ 25°C)
2 mM $MgCl_2$
2 mM DTT
1 mM ATP

Unit Definition

One cohesive end ligation unit (CELU) is the amount of enzyme which gives 50% ligation of 20 pmols of substrate.

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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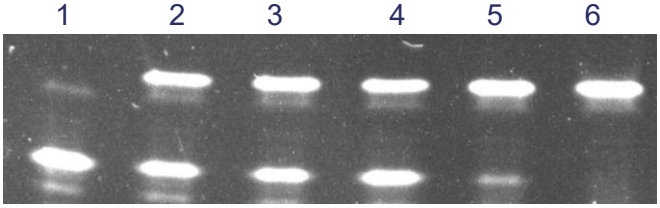
20 pmols (0.22µg) of a double stranded 20-mer with a nick in one strand, composed of a 5' FAM-labelled RNA 8-mer, a 5' phosphorylated DNA 12-mer and a complementary DNA 20-mer is incubated with ligase in 1x Assay Buffer at 37°C for 30 minutes. Samples are then run on a 15 % (w/v) acrylamide/8M urea gel.
1= no ligase ; 2-6= enzyme dilutions 2=1/40, 3=1/20, 4=1/10, 5=1/5, 6=undiluted.



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 >200 U of RNA Ligase 2 for 4 hours at 37°C in the presence of 1 mM ATP showed no detectable conversion of superhelical DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.

Exonuclease assays: (1) >200 U of RNA Ligase 2 incubated with a 20 base, internally labelled single-stranded DNA substrate (0.6 µM) for 4 hours at 37°C showed no detectable degradation.
(2) >200 U of RNA Ligase 2 incubated with a 5' FAM-labelled double-stranded 20 base pair DNA substrate (0.6 µM) for 4 hours at 37°C showed no detectable degradation.

RNase assay: >200 U of RNA Ligase 2 when incubated with a 5' FAM labelled single-stranded RNA 20-mer substrate (3.3 µM) for 4 hours at 37°C showed no detectable degradation.

References

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