T4 DNA Ligase

Product Description (Product Number #DNL1001)

The enzyme is supplied at a concentration of ~20 Weiss 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.

Enzyme Buffer

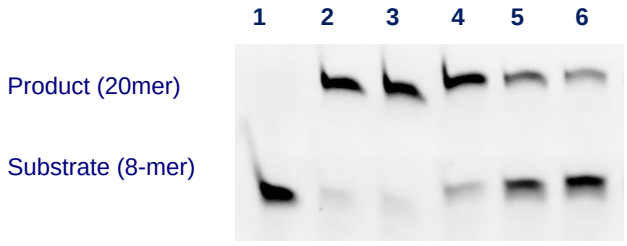
50 mM Tris.HCl (pH 7.5)
100 mM NaCl
1 mM EDTA
1 mM TCEP
50 % (w/v) glycerol
0.1 % Triton X100

Assay Buffer (supplied as 2x stock)

50 mM Tris.HCl (pH 7.5)
10 mM $MgCl_2$
10 mM TCEP
1 mM ATP

Ligation Assay

20 µl of 0.12 µM of a double stranded 20-mer with a nick in one strand, 5' end-labelled on the nicked strand, was incubated with ligase at 16°C for 30 minutes, run on a 15 % (w/v) acrylamide/6M urea gel and visualised using a Fujifilm Fla-7000 Image reader. One cohesive end ligation unit (CELU) is that amount of enzyme which gives 50% ligation.
1=no ligase ; 2-6 enzyme dilutions: 2= 1/10000 ; 3= 1/20000 4= 1/30000 ; 5= 1/40000 ; 6= 1/50000.
100 CELU is equivalent to one Weiss unit.



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 DNA Ligase for 6 hour 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 DNA Ligase was incubated with an internally labelled 20 mer (0.6 µM) for 6 hours at 37°C and no detectable degradation was seen when visualised using a Fujifilm Fla-7000 Image reader.

Reference

Bullard D. R. and Bowater R.P., Biochem.J. (2006) **398**, 135-144
Direct comparison of nick-joining activity of the nucleic acid ligases from bacteriophage T4