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Product Information

T4 DNA Ligase (Rapid)

Catalog Number **KEM0019**
Storage Temperature –20 °C
Unit Size 240,000 U

Product Description
T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5′ phosphate and a 3′ hydroxyl groups of duplex DNA or RNA. The enzyme efficiently joins blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1).

Source of Protein
A recombinant *E. coli* strain carrying the cloned T4 DNA Ligase gene.

Reagent
Supplied at a concentration of 600,000 U/mL in 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, pH 7.5

Supplied with:

2X Rapid Ligation Buffer
Catalog Number KEM0046B
132 mM Tris-HCl, 20 mM $MgCl_2$, 2 mM DTT,
2 mM ATP, 15% PEG 6000, pH 7.6

10X T4 DNA Ligase Buffer
Catalog Number KEM0049B
500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT,
10 mM ATP, pH 7.6

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Unit Definition
1 unit is defined as the amount of DNA Ligase required to join 50% of 100 ng of DNA fragments with cohesive termini in 50 µL 1X DNA Ligase Buffer following a 30 minute incubation at 23°C.

Protocol Rapid Ligation Reaction setup*:

Component	Volume (µL)	Final Concentration
2X Rapid Ligation Buffer	10 µL	1X
Vector	X µL	1-10 ng/µL
Insert	X µL	1-10 ng/µL
T4 DNA Ligase (600 U/µL)	1 µL	30 U/µL
Sterile Water	X µL	N/A
Total Volume	**20 µL**	

* Total reaction volume can be adjusted as needed.

1. Add all of above components to a clean reaction vessel, mix well by pipetting.
2. Incubate at 25 °C for 10 minutes.
3. Immediately purify DNA using PCR clean-up column and elute in ~50 µL.
4. OR - Immediately dilute (at least 1:10, but enough such that 0.1-10 ng of ligation product will be transformed) in TE or water.
5. Transform 0.1-10 ng of ligation product into chemically or electrocompetent cell line that is compatible with vector.

Usage Notes:
One T4 DNA Ligase cohesive end unit is equivalent to approximately 3 cohesive end units as measured with a Lambda-Hind III DNA fragment substrate in 1X T4 DNA Ligase reaction buffer.

One Weiss Unit is approximately equivalent to 22 units.

T4 DNA Ligase is ATP dependent. It is recommended that the reaction buffer be discarded after one year of storage at -20 °C and replaced with fresh buffer to ensure maximum performance. Single-insert ligations are optimal when targeting an insert:vector ratio between 2 and 6. A ratio above 6:1 will promote the insertion of multiple fragments, while dropping below 2:1 will reduce ligation efficiency.

For problematic ligations or if the DNA concentration is unknown, it may be necessary to vary ratios and run multiple ligations.

The presence of PEG at a high concentration will significantly reduce the transformation efficiency of electrocompetent cells. In order to maximize the efficiency of transformation into electrocompetent cells, the following approaches are recommended:

Best: Following ligation, purify the product using a DNA purification spin column and elute in 50 µL of TE. The DNA is now ready for transformation. The final amount of DNA to be transformed should be in the range of 0.1-10 ng.

Better: Dilute ligation product in ddH_20 or TE to reduce the PEG concentration. The final amount of DNA to be transformed should be in the range of 0.1-10 ng.

T4 DNA Ligase in combination with the 2X Rapid Ligation buffer greatly stimulates the rate and efficiency of blunt-end ligation, therefore long incubations (>10 minutes) are NOT recommended and can greatly reduce the transformation efficiency of ligation products. In order to maximize transformation efficiency of the correct insert/vector combination, the protocol provided is recommended.

10X T4 DNA Ligase Buffer does not contain PEG and is compatible with standard ligation protocols which do not specify the use of a rapid/fast/quick format buffer.

Reference

1. Engler, M.J. and Richardson, C.C. (1982) P.D. Boyer (Eds.), The Enzymes, 5, pp. 3. San Diego: Academic Press.

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