Product Information	
T4 DNA Ligase (Rapid)	
Part Number	L6030-HC-L 280225
Concentration	600,000 U/mL
Unit Size	240,000 U
Storage Temperature	-25°C to -15°C

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).

Product Specifications						
L6030						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	**E. coli DNA Contamination**
Units Tested	**n/a**	**n/a**	**6,000**	**6,000**	**6,000**	**6,000**
Specification	>99%	300,000 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

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

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

Molecular weight: 55,292 Daltons

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X DNA Ligase Reaction Buffer and added to 20 µL reactions containing double stranded DNA fragments and 1X DNA Ligase Reaction Buffer. Reactions are incubated for 30 minutes at 23°C, stopped, and analyzed on a 1% agarose gel stained with ethidium bromide.

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

E.coli 16S rDNA Contamination is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in: 10mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol pH 7.5 @ 25ºC.
Supplied with:
2X Rapid Ligation Buffer (B1010): 132mM Tris-HCl, 20mM $MgCl_2$, 2mM DTT, 2mM ATP, 15% PEG 6000 pH 7.6 @ 25ºC.
10X T4 DNA Ligation Buffer (B6030): 500mM Tris-HCl, 100mM $MgCl_2$, 50mM DTT, 10mM ATP pH 7.6 @ 25ºC.

Notes:
One Enzymatics 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 Enzymatics cohesive end 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.
Enzymatics' high-concentration T4 DNA Ligase in combination with the 2X Rapid Ligation buffer greatly stimulates the rate and efficiency 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 following protocol is recommended.
Enzymatics 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.

Usage instructions:
Reaction Set-Up:

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

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.

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