Product Information	
T3 DNA Ligase	
Part Number	L6010L
Concentration	3,000,000 U/mL
Unit Size	900,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: T3 DNA Ligase catalyzes the formation of a phosphodiester bond between a 5' phosphate and a 3' hydroxyl termini in duplex DNA. The enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA. In the absence of 20-30% PEG 6000, T3 DNA Ligase displays a very low efficiency for blunt-ended ligation (1). T3 DNA Ligase retains 95% of its activity in 1.0 M NaCl or KCl, with an optimal concentration of 300 mM (1).

Product Specifications						
L6010						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**30,000**	**30,000**	**30,000**	**30,000**
Specification	>99%	3,000,000 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: A recombinant *E. coli* strain carrying the cloned T3 DNA Ligase gene.

Unit Definition: 1 unit is defined as the amount of T3 DNA Ligase required to ligate 50% of 100 ng DNA fragments with cohesive termini in 30 minutes at 23°C.

Molecular weight: 39.4 KDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X Rapid Ligation Buffer and added to 20 µL reactions containing double stranded DNA fragments and 1X Rapid Ligation Buffer. Reactions were incubated 30 minutes at 23°C (room temp), plunged on ice, 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:
20 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

Supplied with:
2X Rapid Ligation Buffer (B1010): 132 mM Tris-HCl, 20 mM $MgCl_2$, 2 mM DTT, 2 mM ATP, 15% PEG 6000 (pH 7.6 at 25°C)

Usage Instructions: Sticky-end ligation of cut vector and insert

Recommended DNA molar ratio, vector: insert = 1: 3

1. Set up the following reaction mixture in a total volume of 20 µL (add the ligase last):

- 10 µL 2X Rapid Ligation Buffer (B1010)
- 50 ng vector DNA
- 3 fold molar excess of insert DNA
- 1 µL T3 DNA Ligase (L6010)
- Nuclease-free water up to 20 µL

2. Incubate reaction mixture at 25°C for 15-30 minutes.
3. Leave ligated product on ice and transform 1-5 µL of the product into 50 µL of competent cells. Alternatively, the ligation product can be purified using appropriate method, or stored in -20°C.

Notes:
1. T3 DNA Ligase is supplied with 2X Rapid Ligation Buffer for use in ligation reactions. Activity is very low in the absence of PEG (1).
2. Heat inactivation reduces transformation efficiency dramatically.

References:
1. Cai, L. et al. (2004) J. Biochem., 135, 397-403.

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