Product Information	
***E. coli* DNA Ligase**	
Part Number	L6090L
Concentration	10,000 U/mL
Unit Size	2,500 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: *E. coli* DNA ligase catalyzes the phosphodiester bond formation between an adjacent 5' phosphate and a 3' hydroxyl of DNA ends, requiring NAD^+ and Mg^{2+} as cofactors. Ligation of blunt ended DNA is extremely inefficient relative to cohesive DNA end ligation and nick sealing (1).

Product Specifications						
L6090						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**100**	**100**	**100**	**50**
Specification	>99%	20,000 - 28,880 U/mg	<2.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: The gene encoding *E. coli* DNA Ligase expressed from a plasmid in *E. coli*.

Unit Definition: 1 unit is defined as the amount of *E. coli* DNA Ligase required to ligate 50% of 100 ng DNA fragments with cohesive termini in 30 minutes at 25°C.

Molecular weight: 73.6 KDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 20 µL reactions containing double stranded DNA fragments and 1X reaction buffer. Reactions were incubated 30 minutes at 25°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:
10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

Supplied with:
10X *E. coli* DNA Ligase Reaction Buffer (B6090): 300 mM Tris-HCl, 40 mM $MgCl_2$, 10 mM DTT, 260 µM NAD, 0.5 mg/mL BSA (pH 8.0 at 25°C)

Usage Instructions:
1. Set up the following reaction mixture in a total volume of 20 µL:

- 2 µL 10X *E. coli* DNA Ligase Buffer (B6090)
- Up to 5 µg of DNA
- 1 µL (10 U) *E. coli* DNA Ligase (L6090)
- Nuclease-free water up to 20 µL

2. Incubate reaction mixture at 16°C for 30 minutes.
3. Stop the reaction by heat inactivation at 65°C for 20 minutes.

References:
1. Lehman, I.R. (1974) Science, 186, 790-797.

Disclaimer:
Use of this enzyme in certain applications may be covered by patents and may require a license. Purchase of this product does not include a license to perform any patented application; therefore, it is the sole responsibility of the users of the product to determine whether they may be required to engage in a license agreement depending upon the particular application in which the product is used.