Product Information	
LoopLigase® (50U)	
Part Number	L6130L
Concentration	2000 U/mL
Unit Size	50 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: LoopLigase is a thermostable single-stranded ligase capable of circularization of single stranded DNA (ssDNA) or single stranded RNA (ssRNA) templates. It catalyzes the formation of a phosphodiester bond between the terminal 5'-monophosphate and 3'-hydroxyl groups of ssDNA or ssRNA.

Product Specifications						
L6130L						
Assay	**SDS Purity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**Non-specific RNase**
Units Tested	**n/a**	**20 U**	**20 U**	**20 U**	**20 U**	**20 U**
Specification	>99%	<1.0% Released	<1.0% Released	No Conversion	<10 copies	No detectable non-specific RNase

Source of Protein: A recombinant *E. coli* strain carrying the cloned LoopLigase gene.

Unit Definition: One unit is the amount of LoopLigase required to ligate 50% of 0.2 µM of 64 nt linear ssDNA fragments (with 5'-monophosphate and 3'-hydroxyl groups) into circular ssDNA in 15 minutes at 60°C in a 20 µL reaction with 1X LoopLigase buffer.

Molecular weight: 44.05 kDa

Quality Control Analysis:
Protein Concentration (OD_{280}) is determined by OD280 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.

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.

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.

Non-specific RNAse contamination is assessed using a substrate that fluoresces when cleaved by RNAse after incubating for 10 minutes at 37°C. UV excitation at 302nm.

Supplied in: 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol

Supplied with: 5X LoopLigase Buffer (B6130)

Usage instructions: Circularization of ssDNA with 5'-PO_4 and 3'-OH

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

Components	Final Concentration	Volume
Nuclease free water	N/A	14 µL
5X LoopLigase Buffer (B6130)	1X	4 µL
ssDNA substrate	Up to 10 pmol	1 µL
LoopLigase (50U) (L6130L)	2 U	1 µL
	Total Volume =	20 µL

2. Incubate at 60°C for 15 - 60 minutes.
3. Reaction can be stopped by incubation at 85°C for 10 minutes.

Notes:
5X LoopLigase Buffer: To minimize buffer freeze/thaws and ensure maximum performance, it is recommended that the buffer is aliquoted into smaller volumes and stored at -20°C.

Removing un-ligated linear substrates: After the completion of the circularization reaction any remaining un-ligated linear DNA can be removed using exonuclease digestion. To ensure digestion of any possible secondary structures (e.g., hairpins) a combination of Exonuclease I (ssDNA specific) and Exonuclease III (dsDNA specific) can be used. To remove linear RNA from the circularization reaction, RNase R can be used. Circular products of LoopLigase are resistant to the exonucleases.

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.
Related Products:
Exonuclease I (X8010L)
Exonuclease III (X8020