Product Information	
End Repair Mix	
Part Number	Y9140-HC-L
Concentration	1 Rxn/µL
Unit Size	75 Reactions
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: The End-Repair mix converts DNA containing damaged or incompatible 5'- and/or 3'-protruding ends to 5'-phosphorylated, blunt-ended DNA. This high-concentration formulation of the End-Repair Mix is compatible with applications requiring >1 microgram of DNA to be prepared for blunt-end ligation. The conversion to blunt-ended DNA is accomplished by exploiting the 5'→3' polymerase and 3'→5' exonuclease activities of T4 DNA Polymerase (P7080). T4 Polynucleotide Kinase (Y9040) ensures that the ends of the blunt-ended DNA fragments are 5'-phosphorylated for subsequent ligation by T4 DNA Ligase (L6030-HC).

Product Specifications						
Y9140						
Assay	**SDS Purity**	**3'→5' Nuclease**	**5' Phosphorylation**	**5'→3' DNA Synthesis**	**DS Endonuclease**	**E. coli DNA Contamination**
Units Tested	**n/a**	**n/a**	**n/a**	**n/a**	**10µL**	**10µL**
Specification	>99%	Functional	Functional	Functional	No Conversion	<10 copies

Source of Protein: Purified from strains of E. coli that express the recombinant T4 DNA Polymerase, and T4 Polynucleotide Kinase genes, respectively.

Quality Control Analysis:

Functionality is assessed by adding 2µL of End-Repair Mix to a double restriction enzyme digested, dephosphorylated plasmid DNA in 1X reaction buffer containing 0.1mM dNTPs and incubated at 25°C for 30 minutes. Competent cells were transformed with the ligation mixure, plated onto LB/Amp/X-Gal plates and incubated overnight at 37°C. Control reactions consisting of End-Repair Mix without T4 DNA polymerase and/or T4 Polynucleotide Kinase were tested in parallel. The efficiency of the reaction was evaluated by comparing the number of blue and white colonies present in the End-Repair Mix plates to those of the control plates.

Contamination Tests:
Purified free of contaminating endonucleases. In addition, >99% enzyme purity is analyzed by SDS-PAGE, and negligible E.coli genomic DNA is confirmed by qPCR.

Supplied in: 10mM Tris-HCl, 100 mM KCl, 1mM DTT, 0.1mM EDTA, < 0.1% Triton X-100, 50% glycerol pH 7.4 @ 25°C.
Supplied with:
10X End-Repair Buffer (B9140): 1M Tris-HCl, 500mM NaCl, 100mM $MgCl_2$, 50mM DTT, 0.25% Triton X-100, pH 7.5 @ 25°C.
N2060 (1mM dNTPs)
Notes:
ATP is not required because the T4 Polynucleotide Kinase can utilize the deoxynucleotides (dATP and dTTP) used in the reaction as phosphate donors.

Usage Instructions:

1. Purify DNA to be blunted, dissolve in TE buffer.
2. Combine and mix the following components in a sterile tube:

1-19 µL Purified DNA (up to 5 µg)
2.5 µL 10X End-Repair Buffer
2.5 µL 1 mM dNTP mix (N2060)
1-3 µL End-Repair Enzyme Mix
Sterile H_2O to 25 µL
Total Volume: 25 µL

3. Incubate room temperature (25°C) 30 minutes. Inactivate End-Repair Enzyme by heat at 75°C for 20 minutes.
4. Ligation may be performed immediately using Enzymatics Rapid format T4 DNA Ligase (L6030-HC).