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Product Information Sheet *Y9140-LC-L Rev F*

Product Information	
End-Repair Mix (Low Concentration)	
Part Number	Y9140-LC-L 280422
Concentration	1 Rxn/µL
Unit Size	200 Reactions

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
3′→5′ Nuclease	Functional
5′ Phosphorylation	Functional
5′→3′ DNA Synthesis	Functional
DS Endonuclease	10µL = No conversion
***E.coli* DNA Contamination**	10µL <10 copies

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 low-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).

Source of Protein

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

Supplied in

100 mM KCl
10 mM Tris-HCl
0.1 mM EDTA
1 mM DTT
0.1% Triton X-100
50% glycerol
pH 7.4 @ 25°C

Supplied With

B9140 (10X End-Repair Buffer)
N2060 (1 mM dNTPs)

10X End-Repair Buffer (B9140):

1 M Tris-HCl
500 mM NaCl
100 mM $MgCl_2$
50 mM DTT
0.25% Triton X-100
pH 7.5 @ 25°C

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.

Functional Assay

2µL of End-Repair Mix was added 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.

Notes:

1. ATP is not required because the T4 Polynucleotide Kinase can utilize the deoxynucleotides (dATP and dTTP) used in the reaction as phosphate donors.

(additional information on reverse side)

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 1.0 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).

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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.