

# End-It™ DNA End-Repair Kit

Cat. Nos. ER0720 and ER81050

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## 1. Introduction

The End-It™ DNA End-Repair Kit is used to convert DNA with damaged or incompatible 5'-protruding and/or 3'-protruding ends to 5'-phosphorylated, blunt-end DNA for fast and efficient blunt-end ligation into plasmid, cosmid, fosmid, BAC, other cloning vectors, or next-gen DNA sequencing adaptors. End-repaired DNA can be efficiently and rapidly blunt-end ligated into DNA cloning vectors or to next-gen DNA sequencing adaptors using Epicentre's Fast-Link™ DNA Ligation Kits.

## 2. Applications

- Prepare sheared, nebulized, or restriction enzyme digested genomic DNA for ligation of next-gen DNA sequencing adaptors.
- Prepare double-stranded cDNA, produced from cellular RNA transcripts, for ligation of next-gen DNA sequencing adaptors.
- Prepare sheared, nebulized, or restriction enzyme digested DNA for blunt-end ligation into plasmid, cosmid, fosmid, or BAC vectors.
- Prepare DNA amplified by PCR, containing A-overhangs, for efficient and cost-effective blunt-end cloning.

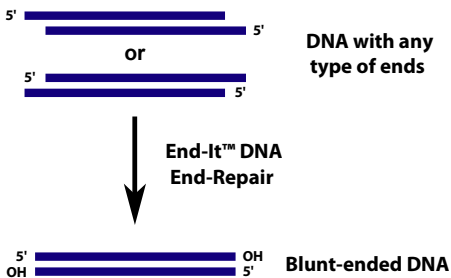
## 3. Product Specifications

**Storage:** Store only at -20°C in a freezer without a defrost cycle.

**Quality Control:** The End-It DNA End-Repair Kit is function-tested by assaying the efficiency of ligation of a PCR product with A-overhangs into a blunt-ended, dephosphorylated plasmid before and after end-repair.

## 4. Kit Contents

Desc.	Quantity
The End-It™ DNA End-Repair Kit is available in 20- and 50-reaction sizes. The 20-reaction size kit contains:	
End-Repair Enzyme Mix	20 µl
End-Repair 10X Buffer	100 µl
ATP	100 µl
dNTPs	100 µl



**Figure 1.** The End-It™ DNA End-Repair Reaction.

## 5. Related Products

The following products are also available:

- Fast-Link™ DNA Ligation Kits
- pIndigoBAC-5 Cloning-Ready Vectors
- MasterPure™ DNA Purification Kits
- GELase™ Agarose Gel-Digesting Preparation
- TransforMax™ EC100™ Electrocompetent *E. coli*

## 6. End-It DNA End-Repair Kit Protocol

1. Purify the DNA to be blunt-ended.

Dissolve the DNA in TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

2. Combine and mix the following components in a microfuge tube (standard reaction).

The standard 50- $\mu$ l reaction will end-repair up to 5  $\mu$ g of DNA.

The reaction can be scaled up as necessary.

1-34 $\mu$ l	DNA to end-repair (up to 5 $\mu$ g)
5 $\mu$ l	10X End-Repair Buffer
5 $\mu$ l	dNTP Mix
5 $\mu$ l	ATP
x $\mu$ l	sterile water to a reaction volume of 49 $\mu$ l
1 $\mu$ l	End-Repair Enzyme Mix
50 $\mu$ l	Total reaction volume

3. Incubate at **room temperature** for 45 minutes.
4. Stop the reaction by heating at 70°C for 10 minutes.

**Note:** *Even after heating at 70°C for 10 minutes, the T4 Polynucleotide Kinase may not be completely inactivated resulting in a high background of non-recombinants due to 5' phosphorylation and self-ligation of the cloning vector during DNA ligation. To reduce background it may be necessary to phenol/chloroform extract the End-It reaction mix and ethanol precipitate the blunt-ended DNA prior to DNA ligation.*

5. The end-repaired DNA can be used for DNA ligation without purification. Perform the blunt-end ligation reaction for 15 minutes to 2 hours at room temperature using Epicentre's Fast-Link DNA Ligation Kit.

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