

Manual

End-It DNA End-Repair Kit

For Research Use Only. Not for use in diagnostic procedures.

End-It™ is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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End-It DNA End-Repair Kit

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 Fast-Link™ DNA Ligation Kits from LGC, Biosearch Technologies.

2. Applications

- Prepare sheared, nebulised, 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, nebulised, 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 designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
End-It DNA End-Repair Kit	20 Rxns	ER0720	End-It Enzyme Mix	E0025-D2	20 µL
			End-It 10X Buffer	SS000272-D1	100 µL
			dNTP Mix (2.5 mM each)	SS000055-D1	100 µL
			ATP (10 mM)	SS000391-D1	100 µL
End-It DNA End-Repair Kit	50 Rxns	ER81050	End-It Enzyme Mix	E0025-D1	50 µL
			End-It 10X Buffer	SS000272-D4	250 µL
			dNTP Mix (2.5 mM each)	SS000055-D2	250 µL
			ATP (10 mM)	SS000391-D2	250 µL

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4. 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.

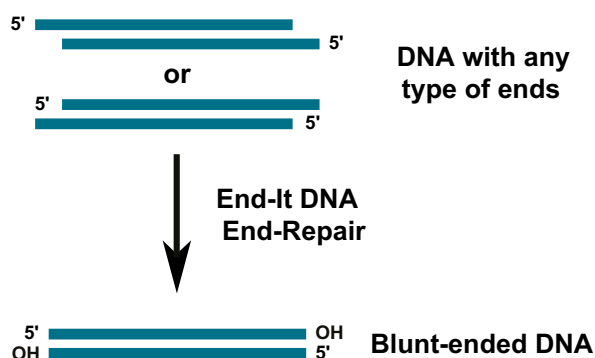


Figure 1. The End-It DNA End-Repair Reaction.

5. 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 µL reaction will end-repair up to 5 µg of DNA.

The reaction can be scaled up as necessary.

1-34	µL DNA to end-repair (up to 5 µg)
5	µL End-It 10X Buffer
5	µL dNTP Mix
5	µL ATP
x	µL sterile water to a reaction volume of 49 µL
1	µL End-It Enzyme Mix
50	µL total reaction volume

3. Incubate at **ambient 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.

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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 ambient temperature using the Fast-Link DNA Ligation Kit from Biosearch Technologies.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team:
techsupport@lgcgroup.com



Integrated tools. Accelerated science.

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biosearchtech.com

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