EPICENTRE® Biotechnologies

End-It™ DNA End-Repair Kit

Cat. No. ***ER0720***

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 or other cloning vectors. The conversion to blunt-end DNA is accomplished by exploiting the 5'→3' polymerase and the 3'→5' exonuclease activities of T4 DNA Polymerase. T4 DNA Polynucleotide Kinase and ATP are also included in the kit for phosphorylation of the 5'-ends of the blunt-ended DNA for subsequent ligation into a cloning vector.

The End-It DNA End-Repair Kit contains reagents sufficient to end-repair up to 100 µg of DNA. The reagents included in the kit are identical to the end-repair reagents provided in the pWEB™ Cosmid Cloning Kit, the pWEB-TNC™ Cosmid Cloning Kit and the EpiFOS™ Fosmid Library Production Kit.

Applications:

- 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.

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.

End-It™ DNA End-Repair Kit Contents

End-Repair Enzyme Mix T4 DNA Polymerase T4 Polynucleotide Kinase	20 µl
End-Repair 10X Buffer (330 mM Tris-acetate [pH 7.8], 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM DTT)	100 µl
ATP (10 mM)	100 µl
dNTPs (2.5 mM each)	100 µl

Product Specifications

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

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*

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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	10X End-Repair Buffer
5 µl	2.5 mM dNTP Mix
5 µl	10 mM ATP
x µl	sterile water to a reaction volume of 49 µl
1 µl	End-Repair Enzyme Mix
50 µl	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.