End-It™ DNA End-Repair Kit

Cat. Nos. ER0720 and ER81050
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

<table>
<thead>
<tr>
<th>Desc.</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-Repair Enzyme Mix</td>
<td>20 µl</td>
</tr>
<tr>
<td>End-Repair 10X Buffer</td>
<td>100 µl</td>
</tr>
<tr>
<td>ATP</td>
<td>100 µl</td>
</tr>
<tr>
<td>dNTPs</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

The End-It™ DNA End-Repair Kit is available in 20- and 50-reaction sizes. The 20-reaction size kit contains:

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

DNA with any type of ends

5’ 

or

5’

End-It™ DNA End-Repair

5’ OH

Blunt-ended DNA

5’ OH
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-μl reaction will end-repair up to 5 μg of DNA.
   The reaction can be scaled up as necessary.
   
<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-34 μl</td>
<td>DNA to end-repair (up to 5 μg)</td>
</tr>
<tr>
<td>5 μl</td>
<td>10X End-Repair Buffer</td>
</tr>
<tr>
<td>5 μl</td>
<td>dNTP Mix</td>
</tr>
<tr>
<td>5 μl</td>
<td>ATP</td>
</tr>
<tr>
<td>x μl</td>
<td>sterile water to a reaction volume of 49 μl</td>
</tr>
<tr>
<td>1 μl</td>
<td>End-Repair Enzyme Mix</td>
</tr>
<tr>
<td>50 μl</td>
<td>Total reaction volume</td>
</tr>
</tbody>
</table>

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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