Thermo SCIENTIFIC

PRODUCT INFORMATION

Fast DNA End Repair Kit

#___ for 50 reactions

Lot: _ Expiry Date: _

Components of the kit

Component	#K0771 50 rxns
End Repair Enzyme Mix	125 µl
10X End Repair Reaction Mix	250 µl

Store at -20°C

www.thermoscientific.com/onebio

Description

The Fast DNA End Repair Kit is used for blunting and phosphorylation of DNA ends in just 5 minutes for subsequent use in blunt-end ligation.

All components of the Kit contain premixed reagents to reduce pipetting steps and provide convenience. The End Repair Enzyme Mix contains an optimized mixture of T4 DNA Polymerase and Klenow Fragment to achieve highly effective blunting of fragmented DNA, and T4 Polynucleotide Kinase for efficient phosphorylation of DNA ends. The 10X End Repair Reaction Mix contains optimized reaction buffer, ATP and dNTPs.

Samples such as fragmented genomic DNA (restriction enzyme digested, nebulized or sonicated), restriction enzyme digested plasmid DNA, double-stranded cDNA and PCR products containing 3'-dA overhangs are all compatible with the kit.

Applications

Blunting and phosphorylation of double-stranded DNA:

- Nebulized DNA
- Sonicated DNA
- RE digested DNA
- cDNA
- PCR products

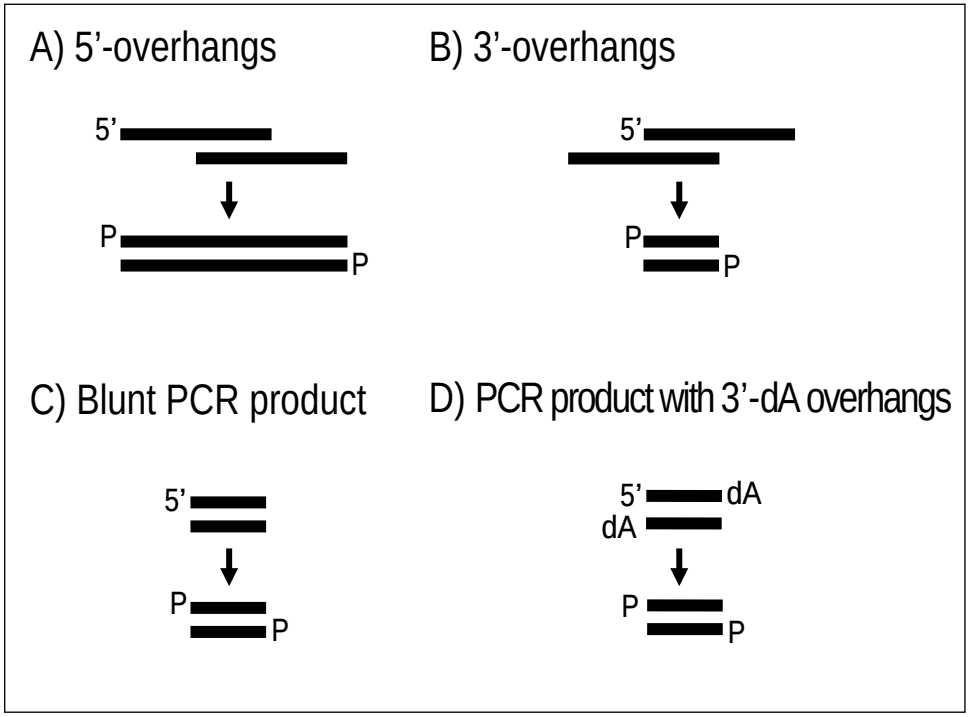
Enzymatic activities of End Repair Enzyme Mix

Activity	Enzyme providing the activity	Type of DNA ends repaired
5'→3' polymerase activity	T4 DNA Polymerase, Klenow Fragment	5'-overhangs
3'→5' exonuclease activity	T4 DNA Polymerase, Klenow Fragment	3'-overhangs
3'-phosphatase activity	T4 PNK	3'-P in sonicated DNA
5'-kinase activity	T4 PNK	Non-phosphorylated DNA (e.g., PCR product)

DNA End Repair Reaction

During the DNA End Repair reaction, fragmented DNA is converted into blunt-end DNA containing 5'-phosphate and 3'-hydroxyl groups. The 5'→3' polymerase activity of the End Repair Enzyme Mix fills-in 5'-protruded DNA ends while 3'→5' exonuclease activity removes 3'-overhangs (Fig. 1). T4 PNK adds 5'-phosphates to ends of unphosphorylated DNA fragments such as PCR products.

Fig. 1. Blunting and phosphorylation of different types of DNA ends.



(continued on reverse page)

PROTOCOL

Important Notes

- Starting material: 0.5-5 µg of DNA fragmented to 100-2500 bp in ≤42.5 µl volume.
- DNA sample must be purified both prior to the DNA End Repair reaction and immediately after the blunting/phosphorylation procedure to remove all enzymes that may damage the blunted DNA ends. We recommend use of spin column based kits such as the Thermo Scientific GeneJET PCR Purification Kit (#K0701, K0702) for both DNA sample purification and re-purification. In-gel DNA samples may be purified using the GeneJET™ Gel Extraction Kit (#K0691, K0692).
- When Thermo Scientific FastDigest restriction enzyme digested plasmid DNA is used for cloning purposes, column purification before the DNA End Repair reaction is optional. However, restriction enzyme must be inactivated.

DNA End Repair Procedure

1. Mix the following components in a sterile microcentrifuge tube on ice:

DNA fragments	0.5-5 µg
10X End Repair Reaction Mix	5 µl
End Repair Enzyme Mix	2.5 µl
Water, nuclease-free (#R0581)	to 50 µl
Total volume	**50 µl**

2. Incubate in a thermal cycler or a heating block for 5 minutes at 20°C.
   Note. Do not exceed 20 min.

3. Purify the repaired blunt-end DNA sample using a spin column based kit such as the GeneJET PCR Purification Kit (#K0701, K0702).

Repaired and purified blunt-end phosphorylated DNA can be stored at −20°C or used immediately in ligation.

For ligation of blunt end DNA we recommend using T4 DNA Ligase (#EL0014).

QUALITY CONTROL

Endo- and Exonucleases

The End Repair Enzyme Mix storage buffer and 10X End Repair Reaction Mix were tested for the absence of contaminating endonucleases and exonucleases in labeled oligonucleotide test.

Functional testing

Blunting efficiency: 3'- and 5'-overhanging oligoduplexes incubated with 2.5 µl of End Repair Enzyme Mix in 50 µl of 1X End Repair Reaction Mix for 5 min and 20 min at 20°C in the presence of [γ-^{33}P]-ATP, then separated on denaturing PAGE and detected by phosphoimaging, resulted in ≥95% of expected bands specific for the blunt end reaction product.

Phosphorylation efficiency: incubation of 5 µg of 200 bp dephosphorylated DNA fragment with 2.5 µl of End Repair Enzyme Mix in 50 µl of 1X End Repair Reaction Mix for 5 min and 20 min at 20°C, followed by column purification, subsequent ligation and analysis on gel resulted in ≥98% of higher molecular weight bands compared to non-ligated DNA.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to http://www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.