Check the product label for actual catalog number, lot and expiry date.

HighEnd™ Repair Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HER0101	40 r of 25 µl	40 µl – HighEnd™ Repair Blend, 1 r/µl 1.5 ml - 10X HighEnd™ Buffer 0.5 ml - 1 mM dNTP Mix	Enzyme blend storage buffer contains Tris, 50% glycerol and other components. 10X HighEnd™ Buffer contains Tris, NaCl, $MgCl_2$, DTT, and other components. *dNTPs serve as building blocks for filling-in reaction and as phosphate donors for phosphorylation.*
HER0105	200 r of 25 µl	5 x 40 µl – HighEnd™ Repair Blend, 1 r/µl 2 x 1.5 ml - 10X HighEnd™ Buffer 3 x 0.5 ml - 1 mM dNTP Mix	

Storage *In the dark at -20°C.*

APPLICATIONS

- Preparation of PCR products, sheared or nebulized DNA, restriction-digested DNA, cDNA for blunt-end ligation
- Conversion of 5'- and/or 3'-protruding ends to 5'-phosphorylated blunt-ended ones

BENEFITS

- Fast and simple blunting and phosphorylation of DNA at once
- Universal - preparing any kind of DNA for blunt-end ligation
- Premium reagents - reproducible results

PRODUCT DETAILS

HighEnd™ Repair Kit is a premium tool designed for rapid and highly efficient DNA end-repair before the ligation reactions. PCR products, sheared or nebulized DNA, restriction-digested DNA and cDNA can be blunted/phosphorylated in a couple of minutes and are ready for an efficient blunt-end ligation and cloning.
The Kit includes HighEnd™ Repair Blend – an optimized mix of T4 DNA Polymerase and T4 Polynucleotide Kinase. The 5'→3' polymerase and 3'→5' exonuclease activities of T4 DNA Polymerase form the blunt-ended DNA. T4 Polynucleotide Kinase phosphorylates 5' DNA ends. The resulting DNA is a high quality blunt-ended substrate for T4 DNA Ligase.

PRODUCT SPECIFICATIONS

- Optimum activity at room temperature, around 25°C
- Inactivation at 75°C for 20 min

Up to 1-5 microgram of the linear DNA can be blunted and phosphorylated in one 20 min reaction. After the thermal inactivation the reaction mixture can be used for blunt-end ligations.

HighEnd™ Repair Kit is an ideal choice for preparing for ligations the PCR products obtained with high fidelity polymerases like ALLin™ HiFi DNA Polymerase (HLE0201).

PROTOCOL

- Check the integrity and the concentration of the DNA prior the reaction.
- Always repurify PCR products before end-repair.
- Thaw and keep reagents on ice. Mix all components well before use.
- The optimal DNA amount in the reaction depends on the lengths of the DNA fragment. For example 1 µg of 1 kb linear DNA has ~3 pmol ends, but 1 µg of 100 bp linear DNA has 10 times more substrate for blunting/phosphorylation; i.e. even 30 pmol DNA ends. Therefore, the shorter is the DNA fragment, the less of it shall be used in micrograms for end-repair or for later ligation reaction.
- For high DNA amounts upscale the reaction accordingly. For example 5 µg of short 100 bp fragment can be end-repaired in 100 µl reaction using 2-5 µl of HighEnd™ Repair Blend.

✓ Prepare a 25 µl reaction:

Linear DNA in TE buffer or water	up to 1 µg (up to 30 pmol ends)
1 µg of 1 kb linear DNA has ~3 pmol ends *1 µg of 0.5 kb linear DNA has ~6 pmol ends* *1 µg of 0.1 kb linear DNA has ~30 pmol ends*	
10X HighEnd™ Buffer	2.5 µl
1 mM dNTP Mix	2.5 µl
DNase-free water (PCR Water, WAT0110)	to 24 µl
HighEnd™ Repair Blend, 1 r/µl	1 µl (max 2 µl)

✓ Mix well; incubate for 20 - 30 min at 25°C.
✓ Inactivate enzymes at 75°C for 20 min and keep cooled in case the ligation is performed immediately or keep frozen in case the ligation is performed later.
✓ Alternatively, re-purify the DNA using PCR clean-up spin column kit, elute in 25 µl of water or TE and keep frozen.
✓ For subsequent ligation and cloning follow the recommendations for Rally™ Rapid Ligation Kit (RLK0101).

IN VITRO RESEARCH USE ONLY

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