DNA Blunting Kit (Cat.# 6025)

Frequently Asked Questions: DNA Blunting Kit

The DNA Blunting Kit allows the quick and efficient conversion of 3′ and 5′ overhangs to blunt ends. This conversion is accomplished simultaneously by the 3′ to 5′ exonuclease and 5′ to 3′ polymerase activities of T4 DNA Polymerase. The resulting blunt-ended DNA can be ligated efficiently into a vector using the same optimized buffer system employed in Takara's DNA Ligation Kits. The reaction can then be used directly in bacterial transformation or in vitro packaging procedures without need for further DNA purification.

Answers to frequently asked questions about the DNA Blunting Kit are presented here. For additional information, refer to the product User Manual and web page.

Q1: How much DNA can be blunted in a single reaction?

A1: About 0.1-0 µg (equivalent to a 5′-DNA termini concentration of about 0.1-10 pmol) of linearized pUC18 DNA (2686 bp) can be blunted. The optimal quantity is 1 to 2 µg.

Q2: Can Ligation Solutions A and B in the DNA Blunting Kit be replaced with the buffers from DNA Ligation Kit Ver. 2?

A2: Ligation solution A and B are also available as DNA Ligation Kit Ver.1. Ligation Kit Ver. 2 is designed for a smaller reaction volumes than Ver. 1 and requires mixing the DNA solution with an equal volume of ligation solution. As such, DNA Ligation Kit Ver. 2 is more susceptible to the influence of components in the DNA solution. Replacing Ligation Solutions A and B in the DNA Blunting Kit with DNA Ligation Kit Ver. 2, therefore, may result in no ligation. If using Ligation Kit Ver. 2, extract the DNA solution with phenol and precipitate with ethanol before using it for the ligation.

Q3: After blunting PCR products with the DNA Blunting Kit, is it necessary to phosphorylate the 5′ end before ligation to a vector?

A3: Unless the primer was phosphorylated at the 5′ end, the PCR product will not have a phosphorylated 5′ end. When performing ligation with a dephosphorylated vector, it is necessary to perform phosphorylation with T4 Polynucleotide Kinase. Following blunting with the DNA Blunting Kit, remove the enzyme completely by phenol/chloroform extraction. Then perform phosphorylation with the T4 Polynucleotide Kinase followed by heat inactivation at 65ºC for 10 min. The reaction mixture can then be used for ligation.

Q4: How can I increase the efficiency of ligation of an insert that has been blunted with the DNA Blunting Kit with a vector?

A4: The ligation efficiency of blunt-end DNA inserts is inherently lower than cohesive-end DNA fragments. To increase efficiency, allow the ligation reaction to proceed overnight. If further improvements are needed, add NaCl to a final concentration of 500 mM to the solution after the ligation. Adding salt may increase transformation efficiency.

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