Tinzyme Co., Limited

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Cre Recombinase

Product Number: DB005

Shipping and Storage

-20℃.

Components

Components	DB005
Cre Recombinase（1U/μL）	25μL
10×Cre Reaction Buffer	1mL

Description

This product is a Cre recombinase obtained by expressing the plasmid encoding the bacteriophage P1 Cre protein in Escherichia coli and purifying and isolating it multiple times. This enzyme does not require energy cofactors, and Cre - mediated recombination quickly reaches equilibrium between the substrate and the reaction product.

Cre recombinase is a type I topoisomerase of bacteriophage P1, catalyzing site-specific recombination of DNA between loxP sites. The loxP recognition element is a 34bp sequence with two 13bp inverted repeat sequences at both ends and an 8bp spacer in the middle for directional purposes. The recombinant product varies depending on the position and relative direction of the loxP site, and two DNA containing a single loxP site will be fused. The DNA between two forward repeating loxP sites will be cleaved in a circular form, while the DNA sequence between two reverse loxP sites will be flipped.

Application

1. DNA cleavage between two loxP sites;
2. Fusion of DNA molecules containing loxP sites;
3. Flipping of DNA sequences between loxP sites.

Usage recommendations

1. Before agarose gel electrophoresis, the Cre recombinase reaction mixture was incubated at 70℃ for 10 minutes.
2. Extending the incubation time will not improve the efficiency of recombination, but may instead lead to the generation of high molecular weight recombinant products.
3. Increasing the amount of Cre recombinase in the reaction will form loxP dependent Cre DNA complexes, thereby inhibiting the recombination reaction.

Quality assurance

After multiple column purifications, SDS-PAGE gel detection showed only a clear and single target band, while PCR method detected no residual E. coli DNA and no contamination by nucleases.

Definition of Activity

The enzyme amount required to generate maximum site-specific recombination of 0.25μg pLox2+control DNA in a 50μL reaction system at 37℃ for 30 minutes is defined as one unit. The largest recombination can be screened for ampicillin resistance by agarose gel electrophoresis or transformation of reactants.

Reaction conditions

1 x Cre reaction buffer [33mM NaCl, 50mM Tris HCl (pH 7.5 stored at 25℃), 10mM $MgCl_2$], incubated at 37℃.

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Thermal deactivation

70℃, 10 minutes.