**Cre Recombinase**

**Description:** Cre Recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between loxP sites (1). The enzyme requires no energy cofactors and Cre-mediated recombination quickly reaches equilibrium between substrate and reaction products (2). The loxP recognition element is a 34 bp sequence comprised of two 13 bp inverted repeats flanking an 8 bp spacer region which confers directionality (3). Recombination products depend on the location and relative orientation of the loxP sites. Two DNA species containing single loxP sites will be fused. DNA between directly repeated loxP sites will be excised in circular form while DNA between opposing loxP sites will be inverted with respect to external sequences.

**Source:** Purified from an *E. coli* strain carrying a plasmid encoding Cre Recombinase from bacteriophage P1 with additional N-terminal Ala and Gly residues (4).

**Applications:**
- Excision of DNA between two loxP sites
- Fusion of DNA molecules containing loxP sites
- Inversion of DNA between loxP sites

**Reagents Supplied with Enzyme:**
- 10X Cre Recombinase Reaction Buffer, Control DNA (linearized pLox2+) (4).

**Quality Control Assays**

**16-Hour Incubation:** Incubation of 10 units of Cre Recombinase with 1 µg of pEX174 RF I DNA (HaeIII digest) in 1X Cre Recombinase Reaction Buffer for 16 hours at 37°C resulted in no detectable exonuclease or endonuclease contamination.

**Exonuclease Activity:** Incubation of 10 units of enzyme with 1 µg sonicated [³H] DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

**Heat Inactivation:** 40 units of enzyme were inactivated by incubation at 70°C for 10 minutes.

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**M0298S**

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• Longer incubation times will not improve recombination, and instead, will likely lead to higher molecular weight recombination products.

• Increasing the amount of Cre Recombinase in the reaction can inhibit recombination by forming $\text{loxP}$ dependent Cre-DNA aggregates.

References:

Figure 1. Cre Recombinase Reaction with $\text{loxP}$ 2+ control substrate. The reactions yields a 20–30 % recombination. Marker M is the 2-Log DNA Ladder (NEB# N0469)