Cre Recombinase

1-800-632-7799
info@neb.com
www.neb.com

M0298S 008140815081

M0298S

RR 37° Yes

50 units **1,000 U/ml** **Lot: 0081408**

RECOMBINANT **Store at –20°C** **Exp: 8/15**

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.

Storage Buffer now includes BSA

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

Supplied in: 15 mM Tris (pH 8.0), 250 mM NaCl, 0.3 mg/ml BSA and 50% glycerol.

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

Reaction Conditions: 1X Cre Recombinase Reaction Buffer. Incubate at 37°C.

1X Cre Recombinase Buffer:
33 mM NaCl
10 mM $MgCl_2$
50 mM Tris-HCl
(pH 7.5 @ 25°C)

Control DNA: Linearized pLox2+ is 3,625 bp in length, with a *loxP* site approximately 400 bp from each end. Between the *loxP* sites lie an origin of replication and an ampicillin-resistance gene. Recombination between these *loxP* sites produces a circular (2,787 bp), ampicillin-resistant plasmid (which migrates at approximately 1.7 kb on a 0.8% agarose gel) and one 838 bp DNA fragment.

Unit Definition: One unit is defined as the amount of enzyme necessary to produce maximal site-specific recombination of 0.25 µg pLox2+ control DNA in 30 minutes at 37°C in a total reaction volume of 50 µl. Maximal recombination is determined by agarose gel analysis and by transformation of reactions followed by selection on ampicillin plates.

Quality Control Assays

16-Hour Incubation: Incubation of 10 units of Cre Recombinase with 1 µg of ϕX174 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 [^{3}H] DNA (10^5 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.

Notes on Use:

- Incubation of the Cre Recombinase reaction mix at 70°C for 10 minutes is recommended before agarose gel analysis.
- Because the Cre Recombinase reaction is an equilibrium reaction, we observe 20–30% recombination on our loxP 2+ control substrate (Fig. 1). This modest yield produces a faint band on an ethidium bromide stained gel and the concomitant reduction in substrate staining intensity.

(See other side)

CERTIFICATE OF ANALYSIS

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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 *loxP* dependent Cre-DNA aggregates.

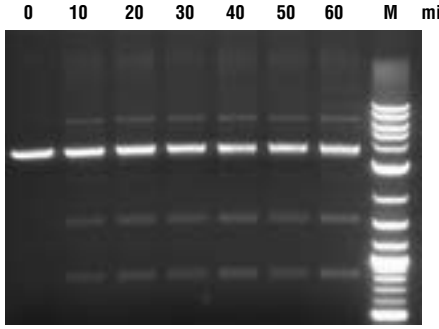

Figure 1. Cre Recombinase Reaction with loxP 2+ control substrate. The reactions yields a 20–30 % recombination. Marker M is the 2-Log DNA Ladder (NEB# N0469)

References:

1. Abremski, K. and R. Hoess (1984) *J. Biol. Chem.* 259, 1509–1514.
2. Abremski, K. et al. (1983) *Cell* 32, 1301–1311.
3. Metzger, D. and Feil, R. (1999) *Curr. Opin. Biotechnol.* 10, 470–476.
4. Cantor, E. and Chong, S. (2001) *Protein Expression and Purification* 22, 135–140.

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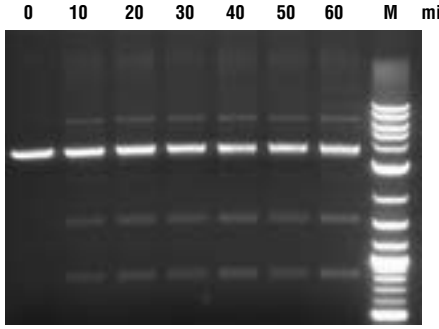

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