Product Specification

Cre Recombinase

Introduction
Cre recombinase (Catalog no. R100-10) is a highly-purified enzyme isolated from 
E. coli expressing recombinant Cre recombinase. The enzyme binds to a specific 34 bp 
sequence (a loxP or loxH site), brings two such sites together, cleaves the DNA, and 
covalently attaches to the DNA. Recombination occurs following two pairs of strand 
exchanges and ligation of the DNAs in a novel (recombinant) form.

Brief Description of Mechanism
A nucleophilic hydroxylated tyrosine initiates the DNA cleavage event by attack on a 
specific phosphodiester bond followed by the covalent attachment of the recombinase to 
the target sequence through a phosphoamino acid bond (Abremski and Hoess, 1992; 
Argos et al., 1986). The reaction does not require any host factors or ATP, but does 
require Mg³⁺ or spermidine for activity (Abremski et al., 1983). In vitro recombination 
between two supercoiled substrates, each containing a loxP site, results in a supercoiled 
dimer. The extent of the reaction is 10-20% under optimal conditions (Abremski and 
Hoess, 1984; Abremski et al., 1983).

Specifications
Volume: 15 µl
Concentration: Refer to label on tube
Storage Buffer: 50 mM Tris HCl pH 7.0, 5 mM EDTA, 1 mM EGTA, 
10 mM β-mercaptoethanol, 20% glycerol

Storage
Store at -20°C or -80°C. When ready for use, thaw on ice and then store at +4°C.

10X Recombinase Buffer
A 10X solution of reaction buffer is provided.
500 mM Tris-HCl, pH 7.5
100 mM MgCl₂
300 mM NaCl
1 mg/ml bovine serum albumin (BSA)

Product Qualification
Purity: >95% homogeneity
Functional Assay: Cre recombinase is qualified using the assay on the next page. The 
donor vector is pUni/lacZ and the acceptor vector is pcDNA3.1-E. Five microliters of the 
recombination reaction is transformed into 50 µl TOP10 One Shot® competent E. coli. 
Twenty-five µl of the transformation reaction is plated on LB plates containing 50 µg/ml 
kanamycin (performed in duplicate). One microliter of Cre recombinase should yield 
>500 blue, kanamycin-resistant transformants.

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Cre Recombinase, Continued

Assay Conditions

If you are using Cre recombinase in conjunction with the Echo™ Cloning System, set up a 20 µl recombination reaction on ice as follows:

- Donor vector (100 ng) x µl
- Acceptor vector (100 ng) y µl
- Cre recombinase 1 µl
- 10X Recombinase Buffer 2 µl

Add sterile water to a final volume of 20 µl

1. Incubate at 37°C for 20 minutes
2. Incubate at 65°C for 5 minutes to inactivate Cre recombinase.
3. Transform 5 µl into competent *E. coli* (TOP10 or equivalent).
4. Plate on selective plates and incubate at 37°C overnight.
5. Recombinant colonies range from 500 to 5000 colonies per plate.

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References


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