XcmI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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R0533S 016120914091

R0533S RR NEB2 37° Yes

1,000 units 5,000 U/ml Lot: 0161209
RECOMBINANT Store at –20°C Exp: 9/14

Recognition Site:

5′. . . C C A N N N N ▼ N N N N T G G . . . 3′
3′. . . G G T N N N N ▲ N N N N A C C . . . 5′

Source: An *E. coli* strain that carries the cloned XcmI gene from *Xanthomonas campestris* (C. Polisson)

2X More Units

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2.

Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 10-fold overdigestion with XcmI, approximately 25% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 50 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases. However, fragments produced by noncanonical cleavage due to star activity may be observed with 5 units of enzyme in similar conditions.

Exonuclease Activity: Incubation of 50 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.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	**100%**
NEBuffer 3	50%
NEBuffer 4	50%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: XcmI produces DNA fragments that have a single-base 3′ extension which are more difficult to ligate than blunt-ended fragments. More efficient ligation can be achieved by using the Quick Ligation Kit (NEB #M2200).

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Conditions of high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

CERTIFICATE OF ANALYSIS

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