MscI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0534S 030121114111

R0534S

NEB4 RR 37° dcm Yes

250 units **5,000 U/ml** **Lot: 0301211**
RECOMBINANT **Store at –20°C** **Exp: 11/14**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned MscI gene from *Micrococcus species* (C. Polisson)

Supplied in: 150 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
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 B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 20-fold overdigestion with MscI, > 95% 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 500 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 500 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.

Endonuclease Activity: Incubation of 20 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	75%
NEBuffer 3	75%
NEBuffer 4	**100%**

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 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 30 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: MscI is an isoschizomer of BalI.

Blocked by overlapping *dcm* methylation. The single MscI site in pBR322 overlaps a *dcm* methylation site; consequently, pBR322 which has been grown in a *dcm*$^{-}$ host should be used for cloning.

Companion Products:

dam^{-}/dcm^{-} Competent *E. coli*

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

CERTIFICATE OF ANALYSIS

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dam-/dcm- Competent E. coli
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CERTIFICATE OF ANALYSIS