MnlI

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

R0163S 061120814081

R0163S

RR NEB4 BSA 37° Yes

500 units **5,000 U/ml** **Lot: 0611208**

RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′. . . C C T C (N)$_7$▼. . . 3′
3′. . . G G A G (N)$_6$▲. . . 5′

Source: An *E. coli* strain that carries the cloned MnlI gene from *Moraxella nonliquefaciens* (ATCC 17953)

New Reaction Buffer

Supplied in: 200 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°CC

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 2-fold overdigestion with MnlI, approximately 50% 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 25 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 100 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	75%
NEBuffer 2	**100%**
NEBuffer 3	50%
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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 5 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: MnlI produces DNA fragments that have a single-base 3′ extension which are more difficult to ligate than blunt-ended fragments.

The cleavage sequence of MnlI has been revised. Brinkley, P., Bautista, D. S., and Graham, F. L. (1991) *Gene* 100, 267–268.

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

= Time-Saver™ Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

MnlI

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

R0163S 061120814081

R0163S

RR NEB4 BSA 37° Yes

500 units 5,000 U/ml Lot: 0611208

RECOMBINANT Store at –20°C Exp: 8/14

Recognition Site:

5′. . . C C T C (N)$_7$▼. . . 3′
3′. . . G G A G (N)$_6$▲. . . 5′

Source: An *E. coli* strain that carries the cloned MnlI gene from *Moraxella nonliquefaciens* (ATCC 17953)

New Reaction Buffer

Supplied in: 200 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°CC

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 2-fold overdigestion with MnlI, approximately 50% 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 25 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 100 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	75%
NEBuffer 2	**100%**
NEBuffer 3	50%
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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 5 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: MnlI produces DNA fragments that have a single-base 3′ extension which are more difficult to ligate than blunt-ended fragments.

The cleavage sequence of MnlI has been revised. Brinkley, P., Bautista, D. S., and Graham, F. L. (1991) *Gene* 100, 267–268.

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

= Time-Saver™ Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS