MlyI

1-800-632-7799
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R0610S 006120914091

R0610S

NEB4 BSA 37° Yes

1,000 units **10,000 U/ml** **Lot: 0061209**

RECOMBINANT **Store at –20°C** **Exp: 9/14**

Recognition Site:

5′...G A G T C $(N)_5$▼...3′
3′...C T C A G $(N)_5$▲...5′

Source: An *E. coli* strain that carries the cloned MlyI gene from *Micrococcus lylae* (NBL 2048)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 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 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 A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with MlyI, approximately 75% 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, approximately 75% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 10 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 30 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 < 0.5% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	50%
NEBuffer 3	25%
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 1.0 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Notes: MlyI is an isoschizomer of PleI that generates blunt-ended DNA fragments.

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

U.S. Patent No. 6,395, 531

CERTIFICATE OF ANALYSIS

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