DdeI

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

R0175S 141120914091

R0175S

1,000 units **10,000 U/ml** **Lot: 1411209**

RECOMBINANT Store at –20°C Exp: 9/14

Recognition Site:

5′. . . C▼TNAG . . . 3′
3′. . . GANT▲C . . . 5′

Source: An *E. coli* strain that carries the cloned DdeI gene from *Desulfovibrio desulfuricans* (NCIB 83120)

More Units

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 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 DdeI, > 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 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 40 units of enzyme with 1 µg sonicated ^3H 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	**100%**
NEBuffer 4	75%

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

Notes: Cleaves single-stranded DNA slowly.

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

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

CERTIFICATE OF ANALYSIS

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NEBuffer 3 100%
NEBuffer 4 75%
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