BmrI

1-800-632-7799
info@neb.com
www.neb.com

R0600S 007121214121

R0600S

50 units **1,000 U/ml** **Lot: 0071212**

RECOMBINANT **Store at –20°C** **Exp: 12/14**

Recognition Site:

5′... A C T G G G $(N)_5$▼... 3′
3′... T G A C C C $(N)_4$ ▲... 5′

Source: An *E. coli* strain that carries the cloned BmrI gene from *Bacillus megaterium* (T. Le)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 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 (HindIII digest) 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 dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 2-fold overdigestion with BmrI, 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, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 4 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 4 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 4% radioactivity.

Endonuclease Activity: Incubation of 1 units of enzyme with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	**100%**
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: Not recommended for digest over 1 hour.

Heat Inactivation: 25 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

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

Overnight digestion with BmrI is not recommended.

Active in the presence of EDTA.

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

BmrI cleaves DNA specifically both in the presence and absence of Mg^{2+} ions in the reaction mixture.

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

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