

BamHI-HF™



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



R3136S 006120814081

R3136S



10,000 units 20,000 U/ml Lot: 0061208

RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5'...GGATCC...3'
3'...CCTAGG...5'

Note: BamHI-HF™ has the same specificity as BamHI (NEB #R0136), but it has been engineered for reduced star activity.

More Units

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Source: An *E. coli* strain that carries the cloned and modified (E163A/E167T) BamHI gene from *Bacillus amyloliquefaciens* H (ATCC 49763)

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

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Quality Controls

Ligation: After 50-fold overdigestion with BamHI-HF, > 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 100 units of BamHI-HF 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 BamHI-HF with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 units of BamHI-HF with 1 µg φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

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Blue/White Screening Assay: An appropriate vector is digested at a unique site within the lacZ^α gene with a 10-fold excess of enzyme. The vector DNA is then ligated, transformed, and plated onto Xgal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, removal of even a single base gives rise to a white colony. Enzyme preparations must produce fewer than 3% white colonies to be Blue/White certified.

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 100%
NEBuffer 2 50%
NEBuffer 3 10%
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.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

Survival in a Reaction: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: No


Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 5 units, pBR322 = 1 unit, LITMUS = 5 units.


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


Requires 5 units to cut pUC19 DNA plasmids or plasmids with similar flanking sequences.

The increased specificity for the BamHI-HF cut site has increased binding of the enzyme to the DNA and the enzyme may remain attached to the DNA during gel electrophoresis. To disrupt binding, add SDS to a final concentration of 0.5%–1% or purify DNA before electrophoresis.

New icons (see www.neb.com for details)

 = Time-Saver™ Qualified

 = indicates that the enzyme has been engineered

 = indicates that the enzyme has reduced star activity

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