HindIII-HF™

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

R3104S 003121014101

R3104S

10,000 units	**20,000 U/ml**	**Lot: 0031210**
RECOMBINANT	**Store at –20°C**	**Exp: 10/14**

Recognition Site:

5′. . . A▼AGCTT . . . 3′
3′. . . TTCGA▲A . . . 5′

Note: HindIII-HF™ has the same specificity as HindIII, but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified HindIII gene from *Haemophilus influenzae* Rd (ATCC 51907)

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 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 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 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 (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Quality Control Assays

Ligation: After 100-fold overdigestion with HindIII-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 400 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.

Endonuclease Assay: Incubation of a 50 µl reaction containing 60 units of HindIII-HF with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of 1,000 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	10%
NEBuffer 2	100%
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.

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°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: LITMUS = 4 units, pBR322 = 2.5 units, pUC19 = 5 units.

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

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

Covered under U.S. Publication No. 2009-0029376

CERTIFICATE OF ANALYSIS

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