NcoI-HF™

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

R3193S 0041210141O1

R3193S

1,000 units **20,000 U/ml** **Lot: 0041210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . C▼CATGG . . . 3′
3′. . . GGTAC▲C . . . 5′

Note: NcoI-HF™ has the same specificity as NcoI (NEB #R0193), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (A2T/R31A) NcoI gene from *Nocardia corallina* (ATCC 19070)

Supplied in: 200 mM NaCl, 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 @ 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, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Controls

Ligation: After 20-fold overdigestion with NcoI-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 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 100 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.

Endonuclease Activity: Incubation of 40 units of enzyme 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.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*$^{\alpha}$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on 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, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 1% white colonies to be Blue/White Certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
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.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)
CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 200 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

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

Companion Products Sold Separately:

NcoI

#R0193S	1,000 units
#R0193L	5,000 units
#R0193T	1,000 units
#R0193M	5,000 units

NcoI-HF™ RE-Mix™

#R5193S	50 reactions

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

U.S. Patent No. 5,202,248

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