



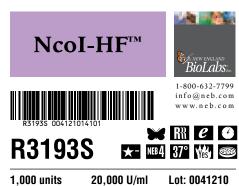
 1,000 units
 20,000 U/ml
 Lot: 0041210

 RECOMBINANT
 Store at -20°C
 Exp: 10/14

## **Recognition Site:**

5′... C<sup>V</sup>C A T G G ... 3′ 3′... G G T A C<sub>4</sub>C ... 5′

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



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**Source:** An *E. coli* strain that carries the cloned and modified (A2T/R31A) Ncol 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  $\mu$ g of  $\lambda$  DNA in 1 hour at 37°C in a total reaction volume of 50  $\mu$ l.

**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)

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# **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  $1-2 \ \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

**16-Hour Incubation:** A 50  $\mu$ I reaction containing 1  $\mu$ g 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  $\mu$ g sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/ $\mu$ g) for 4 hours at 37°C in 50  $\mu$ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 40 units of enzyme with 1  $\mu$ g  $\phi$ X174 RF I DNA for 4 hours at 37°C in 50  $\mu$ I reaction buffer resulted in < 10% conversion to RF II.

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# 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)

# 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:**

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

Ncol-HF<sup>™</sup> RE-Mix<sup>™</sup> #R5193S 50 reactions

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

🅐 = Time-Saver™ Qualified

 $\boldsymbol{\ell}$  = indicates that the enzyme has been engineered

 $\star$  = indicates that the enzyme has reduced star activity

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

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

Ncol-HF<sup>™</sup> RE-Mix<sup>™</sup> #R5193S 50 reactions

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*C* = indicates that the enzyme has been engineered

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

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