

# NgoMIV



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



R0564S 018120814081

## R0564S



**1,000 units 10,000 U/ml Lot: 0181208**  
**RECOMBINANT Store at -20°C Exp: 8/14**

### Recognition Site:

5'... G<sup>▼</sup>CCGGC... 3'  
3'... CGGCC<sup>▲</sup>G... 5'

**Source:** An *E. coli* strain that carries the cloned NgoMIV gene from *Neisseria gonorrhoeae* MS11 (M. So)

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 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 Adenovirus-2 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 DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

### Quality Control Assays

**Ligation:** After 10-fold overdigestion with NgoMIV, > 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 12 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 <sup>3</sup>H DNA (10<sup>5</sup> cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 50 units of enzyme with 1 µg φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 20% 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<sup>x</sup>* 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 3% white colonies to be Blue/White Certified.

### Enzyme Properties

#### Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	50%
NEBuffer 3	10%
NEBuffer 4	<b>100%</b>

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.

**Notes:** NgoMIV is an isoschizomer of NaeI.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

CERTIFICATE OF ANALYSIS

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