

# FatI



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



R0650S 005120714071

**R0650S**

**50 units**      **1,000 U/ml**      **Lot: 0051207**

**RECOMBINANT**    **Store at -20°C**    **Exp: 7/14**

#### Recognition Site:

5'...▼CATG... 3'

3'...GTA C▲... 5'

**Source:** An *E. coli* strain that carries the cloned FatI gene from *Flavobacterium aquatile* NL3 (S.K. Degtyarev)

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 2.

**Reaction Conditions:** 1X NEBuffer 2.  
**Incubate at 55°C.**

**1X NEBuffer 2:**  
50 mM NaCl  
10 mM Tris-HCl  
10 mM MgCl<sub>2</sub>  
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 pUC19 DNA in 1 hour at 55°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 FatI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase at 16°C. Of these ligated fragments, > 95% can be recut.

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of DNA and 10 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 10 units of enzyme with 1 µg sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/µg) for 4 hours at 55°C in 50 µl reaction buffer released < 0.1% radioactivity.

#### Enzyme Properties

##### Activity in NEBuffers:

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

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 0.5 unit is required to digest 1 µg of substrate DNA in 16 hours.

**Heat Inactivation:** 65°C for 20 minutes.

**Note:** FatI is a neoschizomer of NlaIII.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation. Incubation at 37° results in 20% activity.

CERTIFICATE OF ANALYSIS

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