MfeI-HF™

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

R3589S

500 units	**20,000 U/ml**	**Lot: 0041207**
RECOMBINANT	**Store at –20°C**	**Exp: 7/13**

Recognition Site:

5′. . . C▼A A T T G . . . 3′
3′. . . G T T A A▲C . . . 5′

Note: MfeI-HF™ has the same specificity as MfeI (NEB #R0589), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (Q13A/F35Y) MfeI gene from *Mycoplasma fermentas* (N.F. Halden)

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 at 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 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 Controls

Ligation: After 20-fold overdigestion with MfeI-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 35 units of MfeI-HF 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 20 units of MfeI-HF with 1 µg sonicated ^{3}H DNA (10^{5} cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.3% radioactivity.

Endonuclease Activity: Incubation of 70 units of MfeI-HF with 1 µg pUC19 plasmid DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

Blue/White Screening Assay: An appropriate vector is digested at a unique site within the lacZα gene with a 10-fold excess of enzyme. The vector DNA is then ligated, transformed, and plated onto 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, removal of even a single base gives rise to a white colony. Enzyme preparations must produce fewer than 3% white colonies to be Blue/White certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	50%
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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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: 65°C for 20 minutes.

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

Companion Products:

MfeI
#R0589S 500 units
#R0589L 2,500 units

MfeI-HF™ RE-Mix™
#R5589S 25 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

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