

## New England Biolabs Certificate of Analysis

**Product Name:** MfeI-HF<sup>®</sup>  
**Catalog Number:** R3589S  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart<sup>™</sup> Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10199801  
**Expiration Date:** 05/2025  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R3589S/L v3.0

| MfeI-HF <sup>®</sup> Component List |                               |            |                      |
|-------------------------------------|-------------------------------|------------|----------------------|
| NEB Part Number                     | Component Description         | Lot Number | Individual QC Result |
| R3589SVIAL                          | MfeI-HF <sup>®</sup>          | 10194578   | Pass                 |
| B7024AVIAL                          | Gel Loading Dye, Purple (6X)  | 10184699   | Pass                 |
| B6004SVIAL                          | rCutSmart <sup>™</sup> Buffer | 10193043   | Pass                 |

| Assay Name/Specification   | Lot # 10199801 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>A sample of LITMUS38i vector linearized with a 10-fold excess of MfeI-HF <sup>®</sup> , religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.   | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 20 units of MfeI-HF <sup>®</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.             | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 20 units of MfeI-HF <sup>®</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of Lambda DNA and 1 µl of  | Pass           |

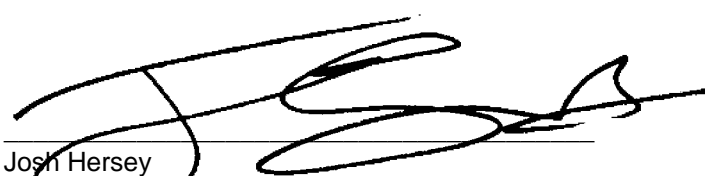
| Assay Name/Specification  | Lot # 10199801 |
|---|----------------|
| <p>MfeI-HF<sup>®</sup> incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>   |                |
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>After a 20-fold over-digestion of Lambda DNA with MfeI-HF<sup>®</sup>, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with MfeI-HF<sup>®</sup>.</p>   | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 60 units of MfeI-HF<sup>®</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>   | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>MfeI-HF<sup>®</sup> is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>  | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>A minimum of 20 units of MfeI-HF<sup>®</sup> is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p> | <b>Pass</b>    |

This product has been tested and shown to be in compliance with all specifications.

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