

New England Biolabs Certificate of Analysis

Product Name: MfeI-HF[®]
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[™] Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.
Packaging Lot Number: 10165620
Expiration Date: 10/2023
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

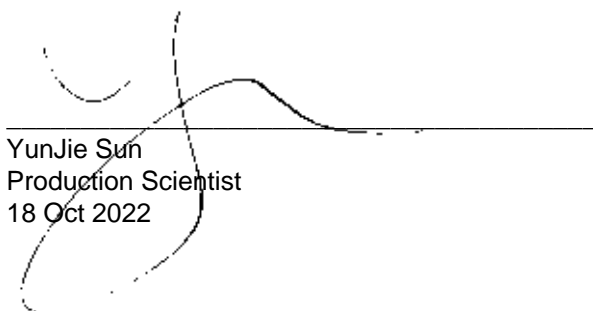
| MfeI-HF [®] Component List | | | |
|-------------------------------------|-------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| R3589SVIAL | MfeI-HF [®] | 10165619 | Pass |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10162784 | Pass |
| B6004SVIAL | rCutSmart [™] Buffer | 10156429 | Pass |

| Assay Name/Specification | Lot # 10165620 |
|--|----------------|
| Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart [™] Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 20 units of MfeI-HF [®] incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart [™] Buffer containing 1 µg of Lambda DNA and 1 µl of MfeI-HF [®] incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis. | Pass |
| Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of Lambda DNA with MfeI-HF [®] , >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with MfeI-HF [®] . | Pass |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart [™] Buffer containing 1 µg of a mixture of single and | Pass |

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|---|----------------|
| <p>double-stranded [³H] E. coli DNA and a minimum of 20 units of MfeI-HF[®] incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.</p> | |
| <p>Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of Lambda DNA and a minimum of 60 units of MfeI-HF[®] incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p> | Pass |
| <p>Protein Purity Assay (SDS-PAGE) MfeI-HF[®] is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p> | Pass |
| <p>qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of MfeI-HF[®] is screened for the presence of E. coli genomic DNA using SYBR[®] 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> | Pass |
| <p>Blue-White Screening (Terminal Integrity) A sample of LITMUS38i vector linearized with a 10-fold excess of MfeI-HF[®], religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.</p> | Pass |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



YunJie Sun
Production Scientist
18 Oct 2022



Michael Tonello
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25 Oct 2022