

## New England Biolabs Certificate of Analysis

**Product Name:** NotI-HF<sup>®</sup>  
**Catalog Number:** R3189M  
**Concentration:** 100,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in rCutSmart<sup>™</sup> Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10181017  
**Expiration Date:** 02/2025  
**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-R3189M v2.0

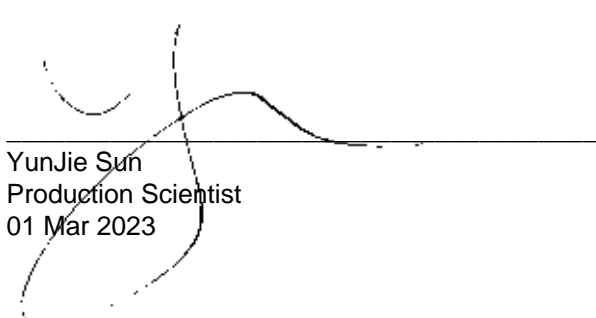
| NotI-HF <sup>®</sup> Component List |                               |            |                      |
|-------------------------------------|-------------------------------|------------|----------------------|
| NEB Part Number                     | Component Description         | Lot Number | Individual QC Result |
| R3189M VIAL                         | NotI-HF <sup>®</sup>          | 10181015   | Pass                 |
| B7024A VIAL                         | Gel Loading Dye, Purple (6X)  | 10175289   | Pass                 |
| B6004S VIAL                         | rCutSmart <sup>™</sup> Buffer | 10175292   | Pass                 |

| Assay Name/Specification  | Lot # 10181017 |
|---|----------------|
| <b>Endonuclease Activity (Nicking)</b><br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of NotI-HF <sup>®</sup> incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.           | <b>Pass</b>    |
| <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 200 units of NotI-HF <sup>®</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | <b>Pass</b>    |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of pBC4 DNA and 1 µl of NotI-HF <sup>®</sup> incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.   | <b>Pass</b>    |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 10-fold over-digestion of pBC4 DNA with NotI-HF <sup>®</sup> , >95% of the DNA fragments  | <b>Pass</b>    |

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|---|----------------|
| <p>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with NotI-HF®.</p>   |                |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBC4 DNA and a minimum of 200 units of NotI-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>  | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>NotI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <p><b>RNase Activity (Extended Digestion)</b><br/>A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of NotI-HF® is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>   | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>A minimum of 20 units of NotI-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> | <b>Pass</b>    |

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

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01 Mar 2023



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