

## New England Biolabs Certificate of Analysis

**Product Name:** BbsI HF  
**Catalog Number:** R3539M  
**Concentration:** 50,000 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:** 10164573  
**Expiration Date:** 09/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 300 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 500 µg/ml rAlbumin, (pH 7.4 @ 25°C)  
**Specification Version:** PS-R3539M v2.0

| BbsI HF Component List |                              |            |                      |
|------------------------|------------------------------|------------|----------------------|
| NEB Part Number        | Component Description        | Lot Number | Individual QC Result |
| R3539MVIAL             | BbsI-HF®                     | 10164570   | Pass                 |
| B7024AVIAL             | Gel Loading Dye, Purple (6X) | 10161525   | Pass                 |
| B6004SVIAL             | rCutSmart™ Buffer            | 10161526   | Pass                 |

| Assay Name/Specification   | Lot # 10164573 |
|--|----------------|
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>           BbsI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | Pass           |
| <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 BbsI-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>   | Pass           |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>           A minimum of 20 units of BbsI-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           |

| Assay Name/Specification   | Lot # 10164573 |
|--|----------------|
| <p><b>Endonuclease Activity (Nicking)</b><br/>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 60 units of BbsI-HF® incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>              | <b>Pass</b>    |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of BbsI-HF® incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>   | <b>Pass</b>    |
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>After a 20-fold over-digestion of Lambda DNA with BbsI-HF®, &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 BbsI-HF®.</p>                                    | <b>Pass</b>    |
| <p><b>Functional Testing (15 minute Digest)</b><br/>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of BbsI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>  | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of BbsI-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>    |

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

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