

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: *10258492*
Expiration Date: *06/2026*
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 |
| R3539M VIAL | BbsI-HF® | 10245427 | Pass |
| B7024A VIAL | Gel Loading Dye, Purple (6X) | 10236426 | Pass |
| B6004S VIAL | rCutSmart™ Buffer | 10249065 | Pass |

| Assay Name/Specification | Lot # 10258492 |
|---|----------------|
| Endonuclease Activity (Nicking) 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 <20% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ H] E. coli DNA and a minimum of 100 units of BbsI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Functional Testing (15 minute Digest) 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. | Pass |
| Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of Lambda DNA with BbsI-HF®, >95% of the DNA | Pass |

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| <p>fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with BbsI-HF®.</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 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> | Pass |
| <p>Protein Purity Assay (SDS-PAGE) BbsI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p> | Pass |
| <p>RNase Activity (Extended Digestion) 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, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p> | Pass |
| <p>qPCR DNA Contamination (E. coli Genomic) 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 |

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

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Nancy Considine
Production Scientist
09 Sep 2024



Michael Tonello
Packaging Quality Control Inspector
10 Sep 2024