

New England Biolabs Certificate of Analysis

Product Name: BbsI-HF®
Catalog Number: R3539L
Concentration: 20,000 U/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.
Packaging Lot Number: 10140772
Expiration Date: 09/2023
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-R3539S/L v2.0

| BbsI-HF® Component List | | | |
|-------------------------|------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| R3539LVIAL | BbsI-HF® | 10119528 | Pass |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10132772 | Pass |
| B6004SVIAL | rCutSmart™ Buffer | 10136929 | Pass |

| Assay Name/Specification | Lot # 10140772 |
|---|----------------|
| Protein Purity Assay (SDS-PAGE) BbsI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| 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. | Pass |
| 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. | 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>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.</p> | Pass |
| <p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H] 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.</p> | Pass |
| <p>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.</p> | Pass |

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

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Penghua Zhang
Production Scientist
08 Mar 2022



Josh Hersey
Packaging Quality Control Inspector
08 Mar 2022