NEW ENGLAND BioLabs Inc.
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Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
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# 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.* |
| *Lot Number:* | *10021240* |
| *Expiration Date:* | *09/2020* |
| *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 BSA, (pH 7.4 @ 25°C)* |
| *Specification Version:* | *PS-R3539S/L v1.0* |

**BbsI-HF® Component List**

| NEB Part Number | Component Description | Lot Number | Individual QC Result |
|---|---|---|---|
| R3539LVIAL | BbsI-HF® | 10020734 | **Pass** |
| B7204SVIAL | CutSmart® Buffer | 10015396 | **Pass** |
| B7024SVIAL | Gel Loading Dye, Purple (6X) | 10018419 | **Pass** |

| Assay Name/Specification | Lot # 10021240 |
|---|---|
| **Exonuclease Activity (Radioactivity Release)**<br>A 50 µl reaction in CutSmart® 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 37ºC releases <0.1% of the total radioactivity. | **Pass** |
| **Functional Testing (15 minute Digest)**<br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda DNA and 1 µl of BbsI-HF incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis. | **Pass** |
| **Ligation and Recutting (Terminal Integrity)**<br>After a 20-fold over-digestion of Lambda DNA with BbsI-HF, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with BbsI-HF. | **Pass** |
| **Non-Specific DNase Activity (16 Hour)**<br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of BbsI-HF incubated for 16 hours at 37ºC results in a DNA pattern free of | **Pass** |

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| Assay Name/Specification | Lot # 10021240 |
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| detectable nuclease degradation as determined by agarose gel electrophoresis. | |
| **Protein Purity Assay (SDS-PAGE)**<br>BbsI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **Endonuclease Activity (Nicking)**<br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 60 units of BbsI-HF incubated for 4 hours at 37ºC results in <20% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |

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

Tony Spear-Alfonso
Production Scientist
09 Aug 2018

Michael Tonello
Packaging Quality Control Inspector
21 Sep 2018