NEW ENGLAND BioLabs Inc.
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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *BsiWI-HF®* |
| *Catalog Number:* | *R3553S* |
| *Concentration:* | *20,000 U/ml* |
| *Unit Definition:* | *One unit is defined as the amount of enzyme required to digest 1 µg of PhiX174 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.* |
| *Packaging Lot Number:* | *10097020* |
| *Expiration Date:* | *01/2023* |
| *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 BSA, (pH 7.4 @ 25°C)* |
| *Specification Version:* | *PS-R3553S/L v1.0* |

| **BsiWI-HF® Component List** | | | |
|---|---|---|---|
| **NEB Part Number** | **Component Description** | **Lot Number** | **Individual QC Result** |
| R3553SVIAL | BsiWI-HF® | 10097021 | **Pass** |
| B7204SVIAL | CutSmart® Buffer | 10092684 | **Pass** |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10089393 | **Pass** |

| **Assay Name/Specification** | **Lot # 10097020** |
|---|---|
| **Endonuclease Activity (Nicking)**<br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 20 units of BsiWI-HF incubated for 4 hours at 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **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 BsiWI-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 PhiX174 DNA and 1 µl of BsiWI-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 PhiX174 DNA with BsiWI-HF, >95% of the DNA | **Pass** |

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| Assay Name/Specification | Lot # 10097020 |
|---|---|
| fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with BsiWI-HF. | |
| **Non-Specific DNase Activity (16 Hour)**<br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of PhiX174 DNA and a minimum of 100 units of BsiWI-HF incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | **Pass** |
| **Protein Purity Assay (SDS-PAGE)**<br>BsiWI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Penghua Zhang
Production Scientist
28 Jan 2021

Michael Tonello
Packaging Quality Control Inspector
28 Jan 2021