

## New England Biolabs Certificate of Analysis

**Product Name:** BsiWI-HF®  
**Catalog Number:** R3553L  
**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:** 10126791  
**Expiration Date:** 10/2023  
**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-R3553S/L v2.0

| BsiWI-HF® Component List |                              |            |                      |
|--------------------------|------------------------------|------------|----------------------|
| NEB Part Number          | Component Description        | Lot Number | Individual QC Result |
| R3553LVIAL               | BsiWI-HF®                    | 10124088   | Pass                 |
| B7024AVIAL               | Gel Loading Dye, Purple (6X) | 10119053   | Pass                 |
| B6004SVIAL               | rCutSmart™ Buffer            | 10119384   | Pass                 |

| Assay Name/Specification   | Lot # 10126791 |
|--|----------------|
| <b>Non-Specific DNase Activity (16 Hour)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of PhiX174 DNA and a minimum of 100 units of BsiWI-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           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 20-fold over-digestion of PhiX174 DNA with BsiWI-HF®, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with BsiWI-HF®.   | Pass           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of PhiX174 DNA and 1 µl of BsiWI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.  | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b>  | Pass           |

| Assay Name/Specification   | Lot # 10126791 |
|--|----------------|
| <p>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 BsiWI-HF® incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>  |                |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>A minimum of 20 units of BsiWI-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> | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>BsiWI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <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 20 units of BsiWI-HF® incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>   | <b>Pass</b>    |

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](http://www.neb.com/trademarks) for additional information.



Penghua Zhang  
Production Scientist  
03 Nov 2021



Michael Tonello  
Packaging Quality Control Inspector  
03 Nov 2021