

## 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:** 10149750  
**Expiration Date:** 05/2024  
**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®	10149746	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10144740	Pass
B6004SVIAL	rCutSmart™ Buffer	10148729	Pass

Assay Name/Specification	Lot # 10149750
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            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>	Pass
<p><b>Endonuclease Activity (Nicking)</b>            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>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b>            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 BsiWI-HF® incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass

Assay Name/Specification	Lot # 10149750
<p><b>Functional Testing (15 minute Digest)</b> 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> BsiWI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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.</p>	<b>Pass</b>
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of PhiX174 DNA with BsiWI-HF®, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with BsiWI-HF®.</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  
06 May 2022



Michael Tonello  
Packaging Quality Control Inspector  
06 May 2022