**Product Name:** BbsI-HF®
**Catalog Number:** R3539S
**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:** 10086402
**Expiration Date:** 04/2022
**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

<table>
<thead>
<tr>
<th>NEB Part Number</th>
<th>Component Description</th>
<th>Lot Number</th>
<th>Individual QC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3539SVIAL</td>
<td>BbsI-HF®</td>
<td>10071223</td>
<td>Pass</td>
</tr>
<tr>
<td>B7204SVIAL</td>
<td>CutSmart® Buffer</td>
<td>10081174</td>
<td>Pass</td>
</tr>
<tr>
<td>B7024AVIAL</td>
<td>Gel Loading Dye, Purple (6X)</td>
<td>10084971</td>
<td>Pass</td>
</tr>
</tbody>
</table>

### Assay Name/Specification

**Endonuclease Activity (Nicking)**
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 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)**
A 50 µl reaction in CutSmart® 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.

**Functional Testing (15 minute Digest)**
A 50 µl reaction in CutSmart® 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.

**Ligation and Recutting (Terminal Integrity)**
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 16°C. Of these ligated fragments,
### Assay Name/Specification

<table>
<thead>
<tr>
<th>Lot # 10086402</th>
</tr>
</thead>
</table>

- **Non-Specific DNase Activity (16 Hour)**
  - 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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
  - **Pass**

- **Protein Purity Assay (SDS-PAGE)**
  - BbsI-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](http://www.neb.com/trademarks) for additional information.

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Production Scientist  
28 Oct 2020

Michael Tonello  
Packaging Quality Control Inspector  
28 Oct 2020