

## New England Biolabs Product Specification

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|-------------------------------|---|
| <i>Product Name:</i>          | <i>BbsI-HF<sup>®</sup></i>  |
| <i>Catalog #:</i>             | <i>R3539M</i>   |
| <i>Concentration:</i>         | <i>50,000 units/ml</i>  |
| <i>Unit Definition:</i>       | <i>One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer<sup>™</sup> in 1 hour at 37°C in a total reaction volume of 50 µl.</i> |
| <i>Shelf Life:</i>            | <i>24 months</i>  |
| <i>Storage Temp:</i>          | <i>-20°C</i>  |
| <i>Storage Conditions:</i>    | <i>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)</i>   |
| <i>Specification Version:</i> | <i>PS-R3539M v2.0</i>   |
| <i>Effective Date:</i>        | <i>02 May 2022</i>  |

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 60 units of BbsI-HF<sup>®</sup> 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 rCutSmart<sup>™</sup> 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 BbsI-HF<sup>®</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (15 minute Digest)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and 1 µl of BbsI-HF<sup>®</sup> 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<sup>®</sup>, >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 BbsI-HF<sup>®</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of BbsI-HF<sup>®</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Protein Purity Assay (SDS-PAGE)** - BbsI-HF<sup>®</sup> is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 20 units of BbsI-HF<sup>®</sup> is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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  $\leq 1$  *E. coli* genome.

**RNase Activity (Extended Digestion)** - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of BbsI-HF<sup>®</sup> is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

*One or more products referenced in this document may be covered by a 3rd-party trademark.  
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Date 02 May 2022

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