New England Biolabs
Product Specification

Product Name: BamHI-HF®
Catalog #: R3136S/L/V
Concentration: 20,000 units/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart™ Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.

Shelf Life: 24 months
Storage Temp: -20°C
Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R3136S/L/V v2.0
Effective Date: 03 Feb 2022

Assay Name/Specification (minimum release criteria)

**Blue-White Screening (Terminal Integrity)** - A sample of pUC19 vector linearized with a 10-fold excess of BamHI-HF®, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

**Ligation and Recutting (Terminal Integrity)** - After a 50-fold over-digestion of Lambda DNA with BamHI-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 BamHI-HF®.

**endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of BamHI-HF® incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - 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 BamHI-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 rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of BamHI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of BamHI-HF® 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)** - BamHI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
New England Biolabs
Product Specification

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>qPCR DNA Contamination (E. coli Genomic)</strong> - A minimum of 20 units of BamHI-HF® is screened for the presence of <em>E. coli</em> genomic DNA using SYBR® Green qPCR with primers specific for the <em>E. coli</em> 16S rRNA locus. Results are quantified using a standard curve generated from purified <em>E. coli</em> genomic DNA. The measured level of <em>E. coli</em> genomic DNA contamination is ≤ 1 <em>E. coli</em> genome.</td>
</tr>
</tbody>
</table>

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.

Derek Robinson
Director, Quality Control

Date: 03 Feb 2022