New England Biolabs
Product Specification

Product Name: BamHI-HF®
Catalog #: R3136T/M
Concentration: 100,000 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-R3136T/M 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.

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.

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®.

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.
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<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<td>qPCR DNA Contamination (E. coli Genomic) - A minimum of 20 units of BamHI-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.</td>
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One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Derek Robinson
Director, Quality Control

Date: 03 Feb 2022