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New England Biolabs Product Specification

BamHI-HF®
R3136S/L/V
20,000 units/ml
One unit is defined as the amount of enzyme required to digest 1 μ g of Lambda DNA in rCutSmart TM Buffer in 1 hour at 37°C in a total reaction volume of 50 μ l.
24 months
-20°C
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)
PS-R3136S/L/V v2.0
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 \mathbb{R} , 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 rCutSmartTM 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 μ l reaction in rCutSmartTM Buffer containing 1 μ g 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 μ l reaction in rCutSmartTM Buffer containing 1 μ g of Lambda DNA and 1 μ l 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 μ l reaction in rCutSmartTM Buffer containing 1 μ g 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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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.

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Date 03 Feb 2022

Derek Robinson Director, Quality Control



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