

New England Biolabs Product Specification

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| Product Name: | <i>BstEII-HF</i> [®] |
| Catalog #: | R3162S/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-R3162S/L/V v2.0 |
| Effective Date: | 03 Feb 2022 |

Assay Name/Specification (minimum release criteria)

Ligation and Recutting (Terminal Integrity) - After a 10-fold over-digestion of Lambda DNA with *BstEII-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 *BstEII-HF*[®].

Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of *BstEII-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 60 units of *BstEII-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 *BstEII-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 60 units of *BstEII-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) - *BstEII-HF*[®] is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of *BstEII-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

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