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New England Biolabs Certificate of Analysis

Product Name: BstEII-HF®
Catalog Number: R3162L
Concentration: 20,000 U/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.

Packaging Lot Number: 10159950
Expiration Date: 08/2024
Storage Temperature: -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

BstEll-HF® Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R3162LVIAL	BstEII-HF®	10159949	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10163561	Pass	
B6004SVIAL	rCutSmart™ Buffer	10161526	Pass	

Assay Name/Specification	Lot # 10159950
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.	Pass
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.	Pass
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®.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of	Pass



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Assay Name/Specification	Lot # 10159950
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.	Pass
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.	Pass
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%	Pass

This product has been tested and shown to be in compliance with all specifications.

conversion to the nicked form as determined by agarose gel electrophoresis.

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.

Jianying Luo Production Scientist 17 Aug 2022 Michael Tonello

Packaging Quality Control Inspector

05 Oct 2022



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