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New England Biolabs Certificate of Analysis

Product Name: Bsal-HF®v2
Catalog Number: R3733S
Concentration: 20,000 U/ml

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg

of pXba DNA in rCutSmart Buffer in 1 hour at 37°C in a total

reaction volume of 50 μl.

Packaging Lot Number: 10169583
Expiration Date: 08/2024
Storage Temperature: -20°C

Storage Conditions: 20mM Tris-HCl, 300mM NaCl, 0.1mM TCEP, 200 µg/ml rAlbumin, 50%

Glycerol, (pH 9.0 @ 25°C)

Specification Version: PS-R3733S/L v2.0

Bsal-HF®v2 Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R3733SVIAL	Bsal-HF®v2	10156743	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10163561	Pass	
B6004SVIAL	rCutSmart™ Buffer	10165692	Pass	

Assay Name/Specification	Lot # 10169583
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of Bsal-HF®v2 incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of Bsal-HF®v2 is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	Pass
Functional Testing (15 minute Digest) A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of pXba DNA and 1 μl of Bsal-HF®v2 incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release)	Pass



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Assay Name/Specification	Lot # 10169583
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 Bsal-HF®v2 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and a minimum of 60 units of Bsal-HF®v2 incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of Bsal-HF®v2 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
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of pXba DNA with Bsal-HF®v2, >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 Bsal-HF®v2.	Pass
Protein Purity Assay (SDS-PAGE) Bsal-HF®v2 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

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

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 14 Jul 2022 Josh Hersey Packaging Quality Control Inspector

08 Nov 2022



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