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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: 10241907
Expiration Date: 01/2026
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3733SVIAL	Bsal-HF®v2	10222543	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10236230	Pass	
B6004SVIAL	rCutSmart™ Buffer	10237089	Pass	

Assay Name/Specification	Lot # 10241907
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
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 Bsal-HF®v2 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 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
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of pXba DNA with Bsal-HF®v2, >95% of the DNA	Pass



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Assay Name/Specification	Lot # 10241907
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.	
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
Protein Purity Assay (SDS-PAGE) Bsal-HF®v2 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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
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

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.

Ana Egana **Production Scientist** 20 May 2024

Josh Hersey Packaging Quality Control Inspector

20 May 2024



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