NEW ENGLAND BioLabs® Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Product Specification

Product Name:	*BsaI-HF®v2*
Catalog #:	*R3733S/L*
Concentration:	*20,000 units/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.*
Shelf Life:	*24 months*
Storage Temp:	*-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*
Effective Date:	*04 Aug 2022*

Assay Name/Specification (minimum release criteria)
Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of BsaI-HF®v2 incubated for 4 hours at 37°C results in <20% 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 [^{3}H] *E. coli* DNA and a minimum of 100 units of BsaI-HF®v2 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.
Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of pXba DNA with BsaI-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 BsaI-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 BsaI-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.
Protein Purity Assay (SDS-PAGE) - BsaI-HF®v2 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
Functional Testing (15 minute Digest) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and 1 µl of BsaI-HF®v2 incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of BsaI-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.

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Product Specification

Assay Name/Specification (minimum release criteria)
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 BsaI-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.

Date 04 Aug 2022

Derek Robinson
Quality Approver