

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Certificate of Analysis

Product Name:	BbsI-HF®
Catalog #:	R3539S/L
Concentration:	20,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 μ g of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.
<i>Lot</i> #:	0031703
Assay Date:	03/2017
Expiration Date:	3/2019
Storage Temp:	-20°C
Storage Conditions:	300 mM NaCl , 10 mM Tris-HCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol , 500 μ g/ml BSA, (pH 7.4 $@$ 25°C)
Specification Version:	PS-R3539S/L v1.0
Effective Date:	09 Jan 2017

Assay Name/Specification (minimum release criteria)	Lot #0031703
Endonuclease Activity (Nicking) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 60 units of BbsI-HF 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 μ l reaction in CutSmart® Buffer containing 1 μ g of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 100 units of BbsI-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 CutSmart® Buffer containing 1 µg of Lambda DNA and 1 µl of BbsI-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 20-fold over-digestion of Lambda DNA with BbsI-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 BbsI-HF.	Pass
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of BbsI-HF 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) - BbsI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass



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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

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Authorized by Derek Robinson 09 Jan 2017



Stephanie Onet

Inspected by Stephanie Cornelio 21 Mar 2017