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

 $50\,\mu l$.

 Lot #:
 0031705

 Assay Date:
 05/2017

 Expiration Date:
 5/2019

 Storage Temp:
 -20°C

Storage Conditions: 300 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 500 µg/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 #0031705
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 μl reaction in CutSmart® Buffer containing 1 μg 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.

Authorized by Derek Robinson 09 Jan 2017







Inspected by Stephanie Cornelio 11 May 2017

Stephani Onetto