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Fax 978-921-1350
www.neb.com
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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *BssHII* |
| *Catalog Number:* | *R0199L* |
| *Concentration:* | *5,000 U/ml* |
| *Unit Definition:* | *One unit is defined as the amount of enzyme required to digest 1 µg Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.* |
| *Packaging Lot Number:* | *10235068* |
| *Expiration Date:* | *03/2026* |
| *Storage Temperature:* | *-20°C* |
| *Storage Conditions:* | *10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)* |
| *Specification Version:* | *PS-R0199S/L v3.0* |

| **BssHII Component List** | | | |
|---|---|---|---|
| **NEB Part Number** | **Component Description** | **Lot Number** | **Individual QC Result** |
| R0199LVIAL | BssHII | 10235064 | **Pass** |
| B6004SVIAL | rCutSmart™ Buffer | 10229454 | **Pass** |

| **Assay Name/Specification** | **Lot # 10235068** |
|---|---|
| **Blue-White Screening (Terminal Integrity)**<br>A sample of LITMUS28i vector linearized with a 10-fold excess of BssHII, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1.0% white colonies. | **Pass** |
| **Endonuclease Activity (Nicking)**<br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pBR322 DNA and a minimum of 25 units of BssHII incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **Exonuclease Activity (Radioactivity Release)**<br>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 50 units of BssHII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | **Pass** |
| **Functional Testing (15 minute Digest)**<br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of BssHII incubated for 15 minutes at 37ºC results in complete digestion as determined | **Pass** |

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| by agarose gel electrophoresis. | |
| **Ligation and Recutting (Terminal Integrity)**<br>After a 20-fold over-digestion of Lambda DNA with BssHII, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with BssHII. | **Pass** |
| **Non-Specific DNase Activity (16 Hour)**<br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 50 units of BssHII incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | **Pass** |
| **Protein Purity Assay (SDS-PAGE)**<br>BssHII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)**<br>A minimum of 5 units of BssHII 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.

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Production Scientist
21 Mar 2024

Michael Tonello
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21 Mar 2024