

New England Biolabs Product Specification

<i>Product Name:</i>	<i>BssHII</i>
<i>Catalog #:</i>	<i>R0199M</i>
<i>Concentration:</i>	<i>25,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>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)</i>
<i>Specification Version:</i>	<i>PS-R0199M v3.0</i>
<i>Effective Date:</i>	<i>17 Mar 2024</i>

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - 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% white colonies.

Endonuclease Activity (Nicking) - 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.

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 50 units of BssHII 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 Lambda DNA with BssHII, >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 BssHII.

Non-Specific DNase Activity (16 Hour) - 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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - BssHII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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qPCR DNA Contamination (*E. coli* Genomic) - 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.

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Nancy Considine

Date 17 Mar 2024

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Quality Approver

