

## New England Biolabs Certificate of Analysis

**Product Name:** *BssHII (25,000 units/ml)*  
**Catalog Number:** *R0199M*  
**Concentration:** *25,000 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:** *10254019*  
**Expiration Date:** *08/2026*  
**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-R0199M v3.0*

BssHII (25,000 units/ml) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0199MVIAL	BssHII	10254020	Pass
B6004SVIAL	rCutSmart™ Buffer	10250203	Pass

Assay Name/Specification	Lot # 10254019
<p><b>Blue-White Screening (Terminal Integrity)</b>            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 &lt;1% white colonies.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking)</b>            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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Exonuclease Activity (Radioactivity Release)</b>            A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 50 units of BssHII incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Ligation and Recutting (Terminal Integrity)</b>            After a 20-fold over-digestion of Lambda DNA with BssHII, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments,</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10254019
>95% can be recut with BssHII.	
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> BssHII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> 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.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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Production Scientist  
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