

## 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 1 hour at 50°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10100713  
**Expiration Date:** 01/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** 300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml BSA  
**Specification Version:** PS-R0199S/L v2.0

BssHII Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0199LVIAL	BssHII	10097028	Pass
B7204SVIAL	CutSmart® Buffer	10093127	Pass

Assay Name/Specification	Lot # 10100713
<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;1.0% white colonies.</p>	Pass
<p><b>Endonuclease Activity (Nicking)</b>            A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled pBR322 DNA and a minimum of 25 units of BssHII incubated for 4 hours at 50°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b>            A 50 µl reaction in CutSmart™ 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 50°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<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, &gt;95% can be recut with BssHII.</p>	Pass

Assay Name/Specification	Lot # 10100713
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 50 Units of BssHII incubated for 16 hours at 50°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>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;1.0% white colonies.</p>	<b>Pass</b>
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart™ 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 50°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, &gt;95% can be recut with BssHII.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 50 Units of BssHII incubated for 16 hours at 50°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<b>Pass</b>

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

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01 Mar 2021



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