

## New England Biolabs Certificate of Analysis

**Product Name:** BssHII  
**Catalog #:** R0199M  
**Concentration:** 25,000 units/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.  
**Lot #:** 0301410  
**Assay Date:** 10/2014  
**Expiration Date:** 10/2016  
**Storage Temp:** -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-R0199M v1.0  
**Effective Date:**

| Assay Name/Specification (minimum release criteria)   | Lot #0301410 |
|---|--------------|
| <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 <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.   | <b>Pass</b>  |
| <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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.                   | <b>Pass</b>  |
| <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] <i>E. coli</i> DNA and a minimum of 50 units of BssHII incubated for 4 hours at 50°C releases <0.1% of the total radioactivity. | <b>Pass</b>  |
| <b>Ligation and Recutting (Terminal Integrity)</b> - 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.  | <b>Pass</b>  |
| <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.     | <b>Pass</b>  |
| <b>Protein Purity Assay (SDS-PAGE)</b> - BssHII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.  | <b>Pass</b>  |



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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.*



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Authorized by  
Derek Robinson



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Inspected by  
David Hough  
24 Oct 2014

