

## 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 1 hour at 50°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>300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml BSA</i>
<i>Specification Version:</i>	<i>PS-R0199M v2.0</i>
<i>Effective Date:</i>	<i>20 Dec 2013</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 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.

**Exonuclease Activity (Radioactivity Release)** - 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 <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 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.

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

\* 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.



Date 20 Dec 2013

Derek Robinson

Director of Quality Control

