

## New England Biolabs Certificate of Analysis

**Product Name:** *BbsI*  
**Catalog #:** *R0539S/L*  
**Concentration:** *10,000 units/ml*  
**Unit Definition:** *One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.*  
**Lot #:** *0371412*  
**Assay Date:** *12/2014*  
**Expiration Date:** *12/2015*  
**Storage Temp:** *-80 °C*  
**Storage Conditions:** *300 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 300 µg/ml BSA*  
**Specification Version:** *PS-R0539S/L v1.0*  
**Effective Date:** *16 Dec 2014*

Assay Name/Specification (minimum release criteria)	Lot #0371412
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in NEBuffer 2.1 containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of BbsI incubated for 4 hours at 37°C results in <20% 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 NEBuffer 2.1 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 BbsI incubated for 4 hours at 37°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 BbsI, >95% of the DNA fragments can be ligated with T4 DNA ligase in 4 hours at 25°C. Of these ligated fragments, >95% can be recut with BbsI.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 ul reaction in NEBuffer 2.1 containing 1 ug of Lambda DNA and a minimum of 50 units of BbsI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	<b>Pass</b>

\* 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.



Authorized by  
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16 Dec 2014



Inspected by  
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17 Dec 2014

