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## New England Biolabs Certificate of Analysis

Product Name:	BbsI
Catalog #:	R0539S/L
Concentration:	5,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
Lot #:	0361310
Assay Date:	10/2013
Expiration Date:	10/2014
Storage Temp:	-70 °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:	21 Aug 2013

Assay Name/Specification (minimum release criteria)	Lot #0361310
<b>Endonuclease Activity (Nicking)</b> - A 50 $\mu$ l reaction in NEBuffer 2.1 containing 1 $\mu$ g of supercoiled pUC19 DNA and a minimum of 5 units of BbsI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 $\mu$ l reaction in NEBuffer 2.1 containing 1 $\mu$ g 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.	Pass
<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 hours at 25°C. Of these ligated fragments, >95% can be recut with BbsI.	Pass
<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.	Pass

\* 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 21 Aug 2013



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Inspected by Cathy Shea 03 Dec 2013