NEW ENGLAND BioLabs Inc.
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240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
www.neb.com
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New England Biolabs
Certificate of Analysis

Product Name:	*BbsI*
Catalog Number:	*R0539L*
Concentration:	*10,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in NEBuffer r2.1 in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number:	*10168570*
Expiration Date:	*10/2024*
Storage Temperature:	*-20°C*
Storage Conditions:	*300 mM NaCl , 10 mM Tris-HCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol , 500 µg/ml rAlbumin, (pH 7.4 @ 25°C)*
Specification Version:	*PS-R0539S/L v3.0*

BbsI Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
R0539LVIAL	BbsI	10166196	**Pass**
B7024AVIAL	Gel Loading Dye, Purple (6X)	10167588	**Pass**
B6002SVIAL	NEBuffer™ r2.1	10154052	**Pass**

Assay Name/Specification	**Lot # 10168570**
Ligation and Recutting (Terminal Integrity) 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 25ºC. Of these ligated fragments, >95% can be recut with BbsI.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of BbsI is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and a minimum of 50 units of BbsI incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Functional Testing (15 minute Digest)	**Pass**

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Assay Name/Specification	Lot # 10168570
A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and 1 µl of BbsI incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis.	
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 50 units of BbsI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer™ r2.1 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.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer™ r2.1 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.	**Pass**
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 50 units of BbsI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Ligation and Recutting (Terminal Integrity) 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 25ºC. Of these ligated fragments, >95% can be recut with BbsI.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of BbsI is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and a minimum of 50 units of BbsI incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Functional Testing (15 minute Digest) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and 1 µl of BbsI	**Pass**

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Assay Name/Specification	Lot # 10168570
incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis.	

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

YunJie Sun
Production Scientist
28 Sep 2022

Michael Tonello
Packaging Quality Control Inspector
04 Nov 2022