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:	*R0539S*
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:	*10228998*
Expiration Date:	*12/2025*
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
R0539SVIAL	BbsI	10222019	**Pass**
B7024AVIAL	Gel Loading Dye, Purple (6X)	10221467	**Pass**
B6002SVIAL	NEBuffer™ r2.1	10193045	**Pass**

Assay Name/Specification	Lot # 10228998
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**
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of a mixture of single and	**Pass**

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Assay Name/Specification	Lot # 10228998
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.	
Functional Testing (15 minute Digest) 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.	**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 incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis.	**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**
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**
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**
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**
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**
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	**Pass**

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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.	

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
18 Jan 2024

Josh Hersey
Packaging Quality Control Inspector
24 Jan 2024