NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

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 NEBuffer r2.1 in 1 hour at 37°C in a total reaction volume of 50 µl.*
Shelf Life:	*24 months*
Storage Temp:	*-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*
Effective Date:	*03 Nov 2022*

Assay Name/Specification (minimum release criteria)

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.

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.

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 37°C results in complete digestion as determined by agarose gel electrophoresis.

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 25°C. Of these ligated fragments, >95% can be recut with BbsI.

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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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.

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Nancy Considine
Quality Approver

Date 03 Nov 2022