NEW ENGLAND BioLabs® Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Product Specification

Product Name:	*BspEI*
Catalog #:	*R0540S/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 (dam -) in NEBuffer r3.1 in 1 hour at 37°C in a total reaction volume of 50 µl.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-R0540S/L v3.0*
Effective Date:	*15 Jun 2023*

Assay Name/Specification (minimum release criteria)
Blue-White Screening (Terminal Integrity) - A sample of LITMUS38i vector linearized with a 10-fold excess of BspEI, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.
Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 units of BspEI 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™ r3.1 containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 100 units of BspEI 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™ r3.1 containing 1 µg of Lambda dam- DNA and 1 µl of BspEI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.
Ligation and Recutting (Terminal Integrity) - After a 10-fold over-digestion of Lambda dam- DNA with BspEI, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with BspEI.
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda dam- DNA and a minimum of 50 units of BspEI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
Protein Purity Assay (SDS-PAGE) - BspEI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Assay Name/Specification (minimum release criteria)
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 10 units of BspEI 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.

Nancy Considine
Quality Approver

Date 15 Jun 2023