

## New England Biolabs Certificate of Analysis

**Product Name:** BspEI  
**Catalog Number:** R0540L  
**Concentration:** 10,000 U/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.  
**Packaging Lot Number:** 10192897  
**Expiration Date:** 06/2025  
**Storage Temperature:** -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

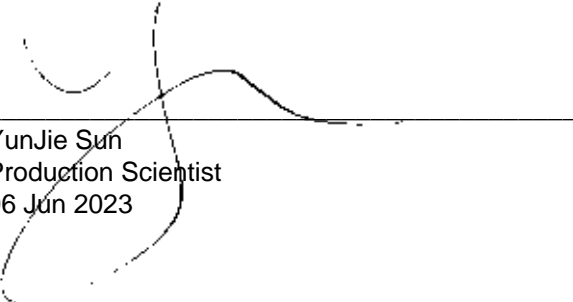
BspEI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0540LVIAL	BspEI	10192528	Pass
B6003SVIAL	NEBuffer™ r3.1	10182162	Pass

Assay Name/Specification	Lot # 10192897
<p><b>Blue-White Screening (Terminal Integrity)</b>            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 &lt;1% white colonies.</p>	Pass
<p><b>Endonuclease Activity (Nicking)</b>            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 &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b>            A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of BspEI incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Functional Testing (15 minute Digest)</b>            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</p>	Pass

Assay Name/Specification	Lot # 10192897
by agarose gel electrophoresis.	
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of Lambda dam- DNA with BspEI, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with BspEI.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> BspEI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> 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.</p>	<b>Pass</b>

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

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