

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>BspEI</i>
<i>Catalog #:</i>	<i>R0540S/L</i>
<i>Concentration:</i>	<i>10,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA (dam<sup>-</sup>) in 1 hour at 37°C in a total reaction volume of 50 µl.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml BSA (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-R0540S/L v2.0</i>
<i>Effective Date:</i>	<i>17 Mar 2022</i>

### 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 [<sup>3</sup>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.

**Ligation and Recutting (Terminal Integrity)** - After a 10-fold over-digestion of Lambda dam<sup>-</sup> 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<sup>-</sup> 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.

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