

## New England Biolabs Certificate of Analysis

**Product Name:** SphI  
**Catalog Number:** R0182L  
**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 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10095466  
**Expiration Date:** 01/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R0182S/L v1.0

SphI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0182LVIAL	SphI	10095467	Pass
B7202SVIAL	NEBuffer™ 2.1	10090560	Pass

Assay Name/Specification	Lot # 10095466
<b>Blue-White Screening (Terminal Integrity)</b> A sample of pUC19 vector linearized with a 10-fold excess of SphI, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer 2.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 30 Units of SphI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer 2.1 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 SphI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of Lambda DNA with SphI, >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 SphI.	Pass

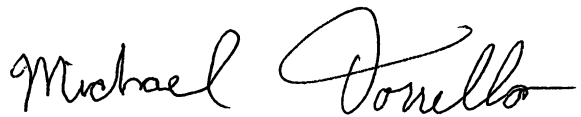
Assay Name/Specification	Lot # 10095466
<p><b>Non-Specific DNase Activity (16 hour)</b> A 50 µl reaction in NEBuffer 2.1 containing 1 µg of Lambda DNA and a minimum of 10 Units of SphI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. NOTE: although no nuclease degradation is detected under these conditions, extended incubations and/or high concentrations of this enzyme may result in star activity. See the product FAQ for recommended reaction conditions for this enzyme.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> SphI is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>

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

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18 Feb 2021



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