

## New England Biolabs Certificate of Analysis

**Product Name:** SphI  
**Catalog Number:** R0182M  
**Concentration:** 80,000 U/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.  
**Packaging Lot Number:** 10172129  
**Expiration Date:** 12/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0182M v2.0

SphI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0182MVIAL	SphI	10172121	Pass
B6002SVIAL	NEBuffer™ r2.1	10154052	Pass

Assay Name/Specification	Lot # 10172129
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            A minimum of 10 units of SphI 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>	Pass
<p><b>Protein Purity Assay (SDS-PAGE)</b>            SphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>Non-Specific DNase Activity (16 hour)</b>            A 50 µl reaction in NEBuffer™ r2.1 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>	Pass

Assay Name/Specification	Lot # 10172129
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer™ r2.1 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 &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 30 units of SphI incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><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 &lt;1% white colonies.</p>	<b>Pass</b>
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of Lambda DNA with SphI, &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 SphI.</p>	<b>Pass</b>

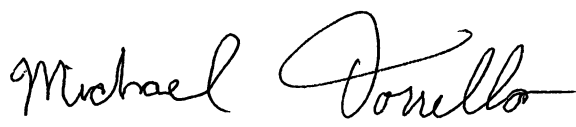
This product has been tested and shown to be in compliance with all specifications.

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08 Dec 2022




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09 Dec 2022