

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: 10194874
Expiration Date: 06/2025
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

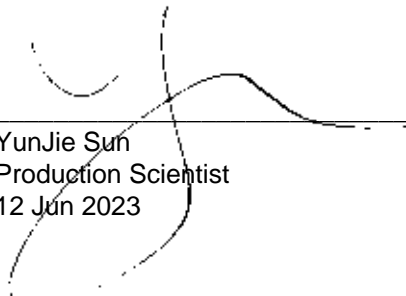
SphI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0182MVIAL	SphI	10194875	Pass
B6002SVIAL	NEBuffer™ r2.1	10193045	Pass

Assay Name/Specification	Lot # 10194874
<p>Blue-White Screening (Terminal Integrity) 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.</p>	Pass
<p>Endonuclease Activity (Nicking) 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 <20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of a mixture of single and double-stranded [³H] 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.</p>	Pass
<p>Ligation and Recutting (Terminal Integrity) 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,</p>	Pass

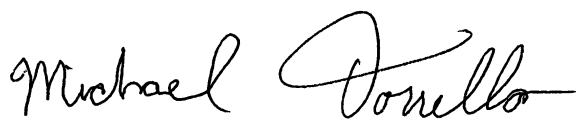
Assay Name/Specification	Lot # 10194874
<p>>95% can be recut with SphI.</p> <p>Non-Specific DNase Activity (16 hour) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and a minimum of 10 units of SphI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation 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>	<p>Pass</p>
<p>Protein Purity Assay (SDS-PAGE) SphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<p>Pass</p>
<p>qPCR DNA Contamination (E. coli Genomic) 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>	<p>Pass</p>

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



YunJie Sun
Production Scientist
12 Jun 2023



Michael Tonello
Packaging Quality Control Inspector
23 Jun 2023