

New England Biolabs Certificate of Analysis

Product Name: *SphI*
Catalog Number: *R0182S*
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 NEBuffer r2.1 in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number: *10249056*
Expiration Date: *07/2026*
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-R0182S/L v2.0*

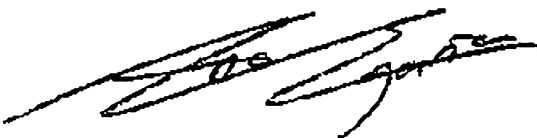
SphI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0182SVIAL	SphI	10245484	Pass
B6002SVIAL	NEBuffer™ r2.1	10231025	Pass

Assay Name/Specification	Lot # 10249056
<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 # 10249056
<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.



Ana Egana
Production Scientist
25 Jun 2024



Michael Tonello
Packaging Quality Control Inspector
25 Jun 2024