NEW ENGLAND BioLabs Inc.
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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 NEBuffer r2.1 in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number:	*10207272*
Expiration Date:	*09/2025*
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-R0182S/L v2.0*

SphI Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
R0182LVIAL	SphI	10206331	**Pass**
B6002SVIAL	NEBuffer™ r2.1	10173667	**Pass**

Assay Name/Specification	**Lot # 10207272**
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.	**Pass**
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.	**Pass**
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.	**Pass**
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 16ºC. Of these ligated fragments,	**Pass**

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Assay Name/Specification	Lot # 10207272
>95% can be recut with SphI.	
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.	**Pass**
Protein Purity Assay (SDS-PAGE) SphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
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.	**Pass**

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
11 Sep 2023

Michael Tonello
Packaging Quality Control Inspector
13 Sep 2023