NEW ENGLAND BioLabs Inc.
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# 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 |
|---|---|
| **Blue-White Screening (Terminal Integrity)**<br>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)**<br>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)**<br>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)**<br>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 # 10194874 |
|---|---|
| >95% can be recut with SphI. | |
| **Non-Specific DNase Activity (16 hour)**<br>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)**<br>SphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)**<br>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.

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Production Scientist
12 Jun 2023

Michael Tonello
Packaging Quality Control Inspector
23 Jun 2023