NEW ENGLAND BioLabs Inc.
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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *SalI-HF®* |
| *Catalog Number:* | *R3138T* |
| *Concentration:* | *100,000 U/ml* |
| *Unit Definition:* | *One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA (HindIII digest) in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.* |
| *Packaging Lot Number:* | *10223621* |
| *Expiration Date:* | *01/2026* |
| *Storage Temperature:* | *-20°C* |
| *Storage Conditions:* | *10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 300 µg/ml rAlbumin, (pH 7.5 @ 25°C)* |
| *Specification Version:* | *PS-R3138T/M v3.0* |

| **SalI-HF® Component List** | | | |
|---|---|---|---|
| **NEB Part Number** | **Component Description** | **Lot Number** | **Individual QC Result** |
| R3138TVIAL | SalI-HF® | 10223619 | **Pass** |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10221112 | **Pass** |
| B6004SVIAL | rCutSmart™ Buffer | 10219598 | **Pass** |

| **Assay Name/Specification** | **Lot # 10223621** |
|---|---|
| **Blue-White Screening (Terminal Integrity)**<br>A sample of pUC19 vector linearized with a 10-fold excess of SalI-HF®, 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 rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of SalI-HF® incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **Exonuclease Activity (Radioactivity Release)**<br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ $^3$H] E. coli DNA and a minimum of 200 units of SalI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | **Pass** |
| **Ligation and Recutting (Terminal Integrity)**<br>After a 50-fold over-digestion of pBC4XS DNA with SalI-HF®, >95% of the DNA | **Pass** |

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| Assay Name/Specification | Lot # 10223621 |
|---|---|
| fragments can be ligated with T4 DNA ligase in 4 hours at 25ºC. Of these ligated fragments, >95% can be recut with SalI-HF®. | |
| **Non-Specific DNase Activity (16 Hour)**<br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBR322 DNA and a minimum of 200 units of SalI-HF® incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | **Pass** |
| **Protein Purity Assay (SDS-PAGE)**<br>SalI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)**<br>A minimum of 20 units of SalI-HF® 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.



Jianying Luo
Production Scientist
09 Jan 2024

Michael Tonello
Packaging Quality Control Inspector
17 Jan 2024