

## New England Biolabs Certificate of Analysis

**Product Name:** *SacI-HF*<sup>®</sup>  
**Catalog Number:** R3156S  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA (*HindIII* digest) in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Lot Number:** 10033499  
**Expiration Date:** 10/2020  
**Storage Temperature:** -20°C  
**Storage Conditions:** 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R3156S/L v2.0

| SacI-HF <sup>®</sup> Component List |                              |            |                      |
|-------------------------------------|------------------------------|------------|----------------------|
| NEB Part Number                     | Component Description        | Lot Number | Individual QC Result |
| R3156SVIAL                          | SacI-HF <sup>®</sup>         | 10026500   | Pass                 |
| B7204SVIAL                          | CutSmart <sup>®</sup> Buffer | 10031564   | Pass                 |
| B7024SVIAL                          | Gel Loading Dye, Purple (6X) | 10021137   | Pass                 |

| Assay Name/Specification   | Lot # 10033499 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>A sample of LITMUS28i vector linearized with a 10-fold excess of SacI-HF <sup>™</sup> , religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.   | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 Units of SacI-HF <sup>™</sup> incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.            | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of SacI-HF <sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 20-fold over-digestion of pXba DNA with SacI-HF <sup>™</sup> , >95% of the DNA fragments   | Pass           |

