

New England Biolabs Product Specification

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| <i>Product Name:</i> | <i>SacI-HF[®]</i> |
| <i>Catalog #:</i> | <i>R3156M</i> |
| <i>Concentration:</i> | <i>100,000 units/ml</i> |
| <i>Unit Definition:</i> | <i>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.</i> |
| <i>Shelf Life:</i> | <i>24 months</i> |
| <i>Storage Temp:</i> | <i>-20°C</i> |
| <i>Storage Conditions:</i> | <i>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)</i> |
| <i>Specification Version:</i> | <i>PS-R3156M v3.0</i> |
| <i>Effective Date:</i> | <i>04 Nov 2022</i> |

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of SacI-HF[®], religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of SacI-HF[®] incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 100 units of SacI-HF[®] incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of Lambda-HindIII DNA and 1 µl of SacI-HF[®] incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of pXba DNA with SacI-HF[®], >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with SacI-HF[®].

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 100 units of SacI-HF[®] incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - SacI-HF[®] is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.



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| qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 20 units of SacI-HF [®] is screened for the presence of <i>E. coli</i> genomic DNA using SYBR [®] Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome. |
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Nancy Considine

Date 04 Nov 2022

Nancy Considine
Quality Approver

