

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>SalI-HF<sup>®</sup></i>
<i>Catalog #:</i>	<i>R3138S/L/E</i>
<i>Concentration:</i>	<i>20,000 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, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 300 µg/ml rAlbumin, (pH 7.5 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-R3138S/L/E v3.0</i>
<i>Effective Date:</i>	<i>12 Jan 2024</i>

### Assay Name/Specification (minimum release criteria)

**Blue-White Screening (Terminal Integrity)** - A sample of pUC19 vector linearized with a 10-fold excess of SalI-HF<sup>®</sup>, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

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

**Protein Purity Assay (SDS-PAGE)** - SalI-HF<sup>®</sup> is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 20 units of SalI-HF<sup>®</sup> is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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.

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of SalI-HF<sup>®</sup> 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<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 200 units of SalI-HF<sup>®</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Ligation and Recutting (Terminal Integrity)** - After a 50-fold over-digestion of pBC4XS DNA with SalI-HF<sup>®</sup>, >95% of the DNA fragments can be ligated with T4 DNA ligase in 4 hours at 25°C. Of these ligated fragments, >95% can be recut with SalI-HF<sup>®</sup>.



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Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBR322 DNA and a minimum of 200 units of Sall-HF® incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
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*Nancy Considine*

Date 12 Jan 2024

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Quality Approver

