

## New England Biolabs Product Specification

<i>Product Name:</i>	SaI-HF <sup>®</sup>
<i>Catalog #:</i>	R3138S/L/V
<i>Concentration:</i>	20,000 units/ml
<i>Unit Definition:</i>	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.
<i>Shelf Life:</i>	24 months
<i>Storage Temp:</i>	-20°C
<i>Storage Conditions:</i>	10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 300 µg/ml BSA, (pH 7.5 @ 25°C)
<i>Specification Version:</i>	PS-R3138S/L/V v2.0
<i>Effective Date:</i>	12 Jul 2021

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

**Blue-White Screening (Terminal Integrity)** - A sample of pUC19 vector linearized with a 10-fold excess of SaI-HF<sup>®</sup>, 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 CutSmart<sup>®</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of SaI-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 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 200 units of SaI-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 SaI-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 SaI-HF<sup>®</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in CutSmart<sup>®</sup> Buffer containing 1 µg of pBR322 DNA and a minimum of 200 units of SaI-HF<sup>®</sup> 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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