

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>NcoI-HF<sup>®</sup></i>
<i>Catalog #:</i>	<i>R3193M</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 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, 200 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-R3193M v2.0</i>
<i>Effective Date:</i>	<i>06 Feb 2023</i>

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

**Blue-White Screening (Terminal Integrity)** - A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI-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 rCutSmart<sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of NcoI-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 100 units of NcoI-HF<sup>®</sup> 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<sup>™</sup> Buffer containing 1 µg of Lambda DNA and 1 µl of NcoI-HF<sup>®</sup> 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 Lambda DNA with NcoI-HF<sup>®</sup>, >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 NcoI-HF<sup>®</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of NcoI-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.

**Protein Purity Assay (SDS-PAGE)** - NcoI-HF<sup>®</sup> 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 NcoI-HF <sup>®</sup> is screened for the presence of <i>E. coli</i> genomic DNA using SYBR <sup>®</sup> 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 $\leq 1$ <i>E. coli</i> genome.
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*Nancy Considine*

Date 06 Feb 2023

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