

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>NotI-HF</i> <sup>®</sup>
<b>Catalog #:</b>	<i>R3189M</i>
<b>Concentration:</b>	<i>100,000 units/ml</i>
<b>Unit Definition:</b>	<i>One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in rCutSmart<sup>™</sup> Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.</i>
<b>Shelf Life:</b>	<i>24 months</i>
<b>Storage Temp:</b>	<i>-20°C</i>
<b>Storage Conditions:</b>	<i>10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)</i>
<b>Specification Version:</b>	<i>PS-R3189M v2.0</i>
<b>Effective Date:</b>	<i>03 Feb 2022</i>

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of NotI-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 NotI-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 pBC4 DNA and 1 µl of NotI-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 10-fold over-digestion of pBC4 DNA with NotI-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 NotI-HF<sup>®</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of pBC4 DNA and a minimum of 200 units of NotI-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)** - NotI-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 NotI-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.



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RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of NotI-HF <sup>®</sup> is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.
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Date 03 Feb 2022

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