

New England Biolabs Certificate of Analysis

Product Name: NotI-HF®
Catalog Number: R3189M
Concentration: 100,000 U/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in rCutSmart™ Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.
Packaging Lot Number: 10198226
Expiration Date: 02/2025
Storage Temperature: -20°C
Storage Conditions: 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)
Specification Version: PS-R3189M v2.0

NotI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3189M VIAL	NotI-HF®	10181015	Pass
B7024A VIAL	Gel Loading Dye, Purple (6X)	10193044	Pass
B6004S VIAL	rCutSmart™ Buffer	10196037	Pass

Assay Name/Specification	Lot # 10198226
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of NotI-HF® incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ H] E. coli DNA and a minimum of 200 units of NotI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBC4 DNA and 1 µl of NotI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
Ligation and Recutting (Terminal Integrity) After a 10-fold over-digestion of pBC4 DNA with NotI-HF®, >95% of the DNA fragments	Pass

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<p>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with NotI-HF®.</p>	
<p>Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBC4 DNA and a minimum of 200 units of NotI-HF® incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) NotI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>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® 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.</p>	Pass
<p>qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of NotI-HF® is screened for the presence of E. coli genomic DNA using SYBR® 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.</p>	Pass

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

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Production Scientist
01 Mar 2023



Michael Tonello
Packaging Quality Control Inspector
17 Aug 2023