

## New England Biolabs Certificate of Analysis


**Product Name:** NotI-HF®  
**Catalog Number:** R3189L  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Lot Number:** 10050226  
**Expiration Date:** 06/2021  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R3189S/L v1.0

NotI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3189LVIAL	NotI-HF®	10046576	Pass
B7204SVIAL	CutSmart® Buffer	10043913	Pass
B7024SVIAL	Gel Loading Dye, Purple (6X)	10043909	Pass

Assay Name/Specification	Lot # 10050226
<p><b>Endonuclease Activity (Nicking)</b>            A 50 µl reaction in CutSmart™ 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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b>            A 50 µl reaction in CutSmart™ 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™ incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Ligation and Recutting (Terminal Integrity)</b>            After a 10-fold over-digestion of pBC4 DNA with NotI-HF™, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with NotI-HF™.</p>	Pass
<p><b>Non-Specific DNase Activity (16 Hour)</b>            A 50 µl reaction in CutSmart™ 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</p>	Pass

Assay Name/Specification	Lot # 10050226
of detectable nuclease degradation as determined by agarose gel electrophoresis.	

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



---

Doreen Duquette  
Production Scientist  
28 May 2019



---

Jay Minichiello  
Packaging Quality Control Inspector  
31 Jul 2019