

## New England Biolabs Certificate of Analysis

**Product Name:** NotI-HF<sup>®</sup>  
**Catalog Number:** R3189S  
**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:** 10039025  
**Expiration Date:** 12/2020  
**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 <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3189SVIAL	NotI-HF <sup>®</sup>	10030791	Pass
B7204SVIAL	CutSmart <sup>®</sup> Buffer	10036665	Pass
B7024SVIAL	Gel Loading Dye, Purple (6X)	10021133	Pass

Assay Name/Specification	Lot # 10039025
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in CutSmart <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.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> 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 NotI-HF <sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> 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> .	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart <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	Pass

Assay Name/Specification	Lot # 10039025
of detectable nuclease degradation as determined by agarose gel electrophoresis.	

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



Stephanie Cornelio  
Production Scientist  
06 Dec 2018



Michael Tonello  
Packaging Quality Control Inspector  
29 Mar 2019