

## 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.  
**Packaging Lot Number:** 10089301  
**Expiration Date:** 08/2022  
**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>	10081007	Pass
B7204SVIAL	CutSmart <sup>®</sup> Buffer	10089407	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10089392	Pass

Assay Name/Specification	Lot # 10089301
<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

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of detectable nuclease degradation as determined by agarose gel electrophoresis.	

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

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