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## New England Biolabs Certificate of Analysis

Product Name:	NotI-HF®
Catalog #:	R3189M
Concentration:	100,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
Lot #:	0041504
Assay Date:	04/2015
Expiration Date:	4/2017
Storage Temp:	-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-R3189M v1.0
Effective Date:	14 Nov 2013

Assay Name/Specification (minimum release criteria)	Lot #0041504
<b>Endonuclease Activity (Nicking)</b> - A 50 μl reaction in CutSmart <sup>TM</sup> Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 100 Units of NotI-HF <sup>TM</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 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 200 units of NotI-HF <sup>TM</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 $TM$ , >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-HFTM.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g of pBC4 DNA and a minimum of 200 Units of NotI-HF <sup>TM</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass

\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

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Authorized by Derek Robinson 14 Nov 2013



Joby F. Unas Inspected by

Inspected by Toby Claus 14 May 2015