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## New England Biolabs Certificate of Analysis

Product Name: NotI-HF®

Catalog #: R3189S/L

Concentration: 20,000 units/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  $\mu l$ .

 Lot #:
 0041703

 Assay Date:
 03/2017

 Expiration Date:
 3/2019

 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-R3189S/L v1.0
Effective Date: 14 Nov 2013

| Assay Name/Specification (minimum release criteria)  | Lot #0041703 |
|--|--------------|
| <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 μl reaction in CutSmart™ Buffer containing 1 μg 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™ 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>TM</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>TM</sup> .                                       | Pass         |
| <b>Non-Specific DNase Activity (16 Hour)</b> - A 50 μl reaction in CutSmart <sup>TM</sup> Buffer containing 1 μg 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         |

<sup>\*</sup> 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.

Authorized by Derek Robinson 14 Nov 2013

ISO 9001
Registered
Quality
Management





Inspected by Stephanie Cornelio 13 Mar 2017

Stephani Unetto