

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

| Product Name:          | NcoI-HF®   |
|------------------------|--|
| Catalog #:             | R3193M   |
| Concentration:         | 100,000 units/ml   |
| Unit Definition:       | One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l. |
| Lot #:                 | 0061804  |
| Assay Date:            | 04/2018  |
| Expiration Date:       | 4/2020   |
| Storage Temp:          | -20°C  |
| Storage Conditions:    | 200 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-R3193M v1.0   |
| Effective Date:        | 29 Oct 2013  |

| Assay Name/Specification (minimum release criteria)  | Lot #0061804 |
|--|--------------|
| <b>Blue-White Screening (Terminal Integrity)</b> - A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI-HF <sup>TM</sup> , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.  | Pass         |
| <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 20 Units of NcoI-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 $\mu$ 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 100 units of NcoI-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 20-fold over-digestion of Lambda DNA with NcoI-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 NcoI-HF <sup>TM</sup> .   | 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 Lambda DNA and a minimum of 100 Units of NcoI-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         |
| <b>Protein Purity Assay (SDS-PAGE)</b> - NcoI-HF <sup>®</sup> is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.  | Pass         |



R3193M Lot: 0061804 Page 1 of 2



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\* 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 29 Oct 2013



Stephanie Onet

Inspected by Stephanie Cornelio 13 Apr 2018