

be INSPIRED *drive* DISCOVERY *stay* GENUINE

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: | Ncol-HF® |
|------------------------|--|
| Catalog Number: | R3193S |
| Concentration: | 20,000 U/ml |
| Unit Definition: | One unit is defined as the amount of enzyme required to digest 1 μ g of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 μ l. |
| Packaging Lot Number: | 10110131 |
| Expiration Date: | 12/2022 |
| Storage Temperature: | -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-R3193S/L v1.0 |

| Ncol-HF® Component List | | | | |
|-------------------------|------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R3193SVIAL | Ncol-HF® | 10094621 | Pass | |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10105817 | Pass | |
| B6004SVIAL | rCutSmart™ Buffer | 10108730 | Pass | |

| Assay Name/Specification | Lot # 10110131 |
|---|----------------|
| Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI-HF [™] , religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies. | Pass |
| Endonuclease Activity (Nicking) A 50 µl reaction in CutSmart [™] Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 Units of Ncol-HF [™] incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart [™] Buffer containing 1 µg of a mixture of single and double-stranded [³ H] E. coli DNA and a minimum of 100 units of Ncol-HF [™] incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of Lambda DNA with Ncol-HF™, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated | Pass |





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| Assay Name/Specification | Lot # 10110131 |
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| fragments, >95% can be recut with NcoI-HF™. | |
| Non-Specific DNase Activity (16 Hour) A 50 µl reaction in CutSmart [™] Buffer containing 1 µg of Lambda DNA and a minimum of 100 Units of Ncol-HF [™] incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass |
| Protein Purity Assay (SDS-PAGE) Ncol-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Penghua Zhang

Penghua Zhang Production Scientist 28 Jun 2021

Mich

Michael Tonello Packaging Quality Control Inspector 28 Jun 2021

