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## New England Biolabs Certificate of Analysis

Product Name: Ncol-HF®

Catalog Number: R3193M

Concentration: 100,000 U/ml

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg

of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Packaging Lot Number: 10118515
Expiration Date: 08/2023
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-R3193M v1.0

| Ncol-HF® Component List |                              |            |                      |  |
|-------------------------|------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b>  | Component Description        | Lot Number | Individual QC Result |  |
| R3193MVIAL              | Ncol-HF®                     | 10118514   | Pass                 |  |
| B7024AVIAL              | Gel Loading Dye, Purple (6X) | 10114156   | Pass                 |  |
| B6004SVIAL              | rCutSmart™ Buffer            | 10115113   | Pass                 |  |

| Assay Name/Specification  | Lot # 10118515 |
|---|----------------|
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and  | Pass           |
| double-stranded [ ³H] 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.   |                |
| 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           |
| Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of Ncol-HF™, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.                                     | Pass           |
| 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  | Pass           |



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| Assay Name/Specification   | Lot # 10118515 |
|--|----------------|
| of detectable nuclease degradation as determined by agarose gel electrophoresis.   |                |
| 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 fragments, >95% can be recut with Ncol-HF™. | 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.

Pengha Zhang Production Scientist 20 Sep 2021 Michael Tonello

Packaging Quality Control Inspector

20 Sep 2021



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