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New England Biolabs Certificate of Analysis

Product Name: Ncol-HF®
Catalog Number: R3193L
Concentration: 20,000 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: 10149727
Expiration Date: 05/2024
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 | |
| R3193LVIAL | Ncol-HF® | 10149725 | Pass | |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10144740 | Pass | |
| B6004SVIAL | rCutSmart™ Buffer | 10148729 | Pass | |

| Assay Name/Specification | Lot # 10149727 |
|---|----------------|
| 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 |
| 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 |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and 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. | 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% | Pass |



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| Assay Name/Specification | Lot # 10149727 |
|---|----------------|
| conversion to the nicked form as determined by agarose gel electrophoresis. | |
| 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 |
| 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 **Production Scientist**

10 May 2022

Michael Tonello

Packaging Quality Control Inspector

10 May 2022



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