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## New England Biolabs Certificate of Analysis

Product Name: Ncol
Catalog Number: R0193M
Concentration: 50,000 U/ml

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

of Lambda DNA in NEBuffer r3.1 in 1 hour at 37°C in a total reaction

volume of 50 μl.

Packaging Lot Number: 10161989
Expiration Date: 09/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200

μg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0193T/M v2.0

| Ncol Component List    |                              |            |                      |  |
|------------------------|------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b> | Component Description        | Lot Number | Individual QC Result |  |
| R0193MVIAL             | Ncol                         | 10161983   | Pass                 |  |
| B7024AVIAL             | Gel Loading Dye, Purple (6X) | 10153339   | Pass                 |  |
| B6003SVIAL             | NEBuffer™ r3.1               | 10146827   | Pass                 |  |

| Assay Name/Specification   | Lot # 10161989 |
|--|----------------|
| Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 10 units of Ncol 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 is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.   | Pass           |
| Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of Ncol, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.  | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b> After a 5-fold over-digestion of Lambda DNA with Ncol, >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.                                    | Pass           |



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| Assay Name/Specification   | Lot # 10161989 |
|--|----------------|
| Functional Testing (15 minute Digest) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of Ncol incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.   | Pass           |
| qPCR DNA Contamination (E. coli Genomic)  A minimum of 10 units of Ncol is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome. | Pass           |
| Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 units of Ncol incubated for 4 hours at 37°C results in <50% conversion to the nicked form as determined by agarose gel electrophoresis.  | Pass           |
| Exonuclease Activity (Radioactivity Release) A 50 μl reaction in NEBuffer <sup>™</sup> r3.1 containing 1 μg of a mixture of single and double-stranded [ ³H] E. coli DNA and a minimum of 50 units of Ncol incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.  | 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.

YunJie Sun \
Production Scientist

23 Aug 2022

Michael Tonello

Packaging Quality Control Inspector

11 Oct 2022



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