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New England Biolabs Certificate of Analysis

Product Name: Ncol
Catalog Number: R0193L
Concentration: 10,000 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.

Lot Number: 1003131
Expiration Date: 12/2020
Storage Temperature: -20°C

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50%

Glycerol, 200 µg/ml BSA

Specification Version: PS-R0193S/L v1.0

| Ncol Component List | | | | |
|------------------------|------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R0193LVIAL | Ncol | 10031318 | Pass | |
| B7203SVIAL | NEBuffer™ 3.1 | 10021113 | Pass | |
| B7024SVIAL | Gel Loading Dye, Purple (6X) | 10021135 | Pass | |

| Assay Name/Specification | Lot # 10031317 |
|--|----------------|
| Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of Ncol, religated | Pass |
| and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies. | |
| Endonuclease Activity (Nicking) | Pass |
| A 50 μl reaction in NEBuffer 3.1 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. | |
| Exonuclease Activity (Radioactivity Release) | Pass |
| A 50 µl reaction in NEBuffer 3.1 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. | |
| Ligation and Recutting (Terminal Integrity) | Pass |
| 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% | |



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| Assay Name/Specification | Lot # 10031317 |
|--|----------------|
| can be recut with Ncol. | |
| Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer 3.1 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 |

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

Tony Spear-Alfonso **Production Scientist**

26 Nov 2018

Josh Hersey

Packaging Ou 14 Dec 2018 ality Control Inspector



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