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## New England Biolabs Certificate of Analysis

Product Name: Ncol
Catalog Number: R0193T
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: 10247913
Expiration Date: 06/2026
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	
R0193TVIAL	Ncol	10245405	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10236423	Pass	
B6003SVIAL	NEBuffer™ r3.1	10237086	Pass	

Assay Name/Specification	Lot # 10247913
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
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™ 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
Functional Testing (15 minute Digest) A 50 μl reaction in NEBuffer™ r3.1 containing 1 μg of Lambda DNA and 1 μl of Ncol	Pass



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Assay Name/Specification	Lot # 10247913
incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	
Ligation and Recutting (Terminal Integrity) 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
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)	Pass
Ncol is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	
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

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.

Ana Egana Production Scientist 12 Jun 2024 Michael Tonello

Packaging Quality Control Inspector

12 Jun 2024



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