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## New England Biolabs Certificate of Analysis

Product Name: Ncol-HF®
Catalog Number: R3193S
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 rCutSmart Buffer in 1 hour at 37°C in a total

reaction volume of 50 μl.

Packaging Lot Number: 10213813
Expiration Date: 09/2025
Storage Temperature: -20°C

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

200 μg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R3193S/L v2.0

Ncol-HF® Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3193SVIAL	Ncol-HF®	10206333	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10204842	Pass	
B6004SVIAL	rCutSmart™ Buffer	10207418	Pass	

Assay Name/Specification	Lot # 10213813
Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI-HF®, 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 rCutSmart™ 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
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ 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
Functional Testing (15 minute Digest) A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of Lambda DNA and 1 μl of	Pass



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Assay Name/Specification	Lot # 10213813
Ncol-HF® incubated for 15 minutes at 37°C results in complete digestion 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
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ 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
Protein Purity Assay (SDS-PAGE) Ncol-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of Ncol-HF® 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.

YunJie Suń Production Scientist

08 **Ş**ép 2023

Josh Hersey

Packaging Quality Control Inspector

26 Nov 2023



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