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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: 10065413
Expiration Date: 11/2021
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3193LVIAL	Ncol-HF®	10059042	Pass	
B7204SVIAL	CutSmart® Buffer	10068803	Pass	
B7024SVIAL	Gel Loading Dye, Purple (6X)	10059231	Pass	

Assay Name/Specification	Lot # 10065413
Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of Ncol-HF™,	Pass
religated 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 CutSmart <sup>™</sup> Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 20 Units of Ncol-HF <sup>™</sup> incubated for 4 hours at 37°C results in <10%	
conversion to the nicked form as determined by agarose gel electrophoresis.	
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and	Pass
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.	
Ligation and Recutting (Terminal Integrity)	Pass
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	. 300



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Assay Name/Specification	Lot # 10065413
fragments, >95% can be recut with Ncol-HF™.	
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
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.

Stephanie Cornelio **Production Scientist** 

13 Nov 2019

Jay Minichiello

Packaging Quality Control Inspector

10 Mar 2020



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