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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 1 hour at 37°C in a total reaction volume of 50 μl.

Packaging Lot Number: 1013772
Expiration Date: 12/2023
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	
R3193SVIAL	Ncol-HF®	10131071	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10131976	Pass	
B6004SVIAL	rCutSmart™ Buffer	10132778	Pass	

Assay Name/Specification	Lot # 10137726
Protein Purity Assay (SDS-PAGE) Ncol-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
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 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
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart™ 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



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Assay Name/Specification	Lot # 10137726
Endonuclease Activity (Nicking) A 50 μl reaction in CutSmart™ 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
Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of Ncol-HF™, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies	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.

Pengha Zhang Production Scientist 17 Feb 2022 Michael Tonello

Packaging Quality Control Inspector

17 Feb 2022



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