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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: 10131072
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)	10126634	Pass	
B6004SVIAL	rCutSmart™ Buffer	10126633	Pass	

Assay Name/Specification	Lot # 10131072
Endonuclease Activity (Nicking) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and	Pass
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.	
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
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
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	Pass



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Assay Name/Specification	Lot # 10131072
of detectable nuclease degradation 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
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.

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 Dec 2021 Michael Tonello

Packaging Quality Control Inspector

17 Dec 2021



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