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New England Biolabs Certificate of Analysis

Product Name: HindIII

Catalog Number: R0104L

Concentration: 20,000 U/mI

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg

of Lambda DNA in NEBuffer r2.1 in 1 hour at 37°C in a total reaction

volume of 50 μl.

Packaging Lot Number: 10176929
Expiration Date: 01/2025
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol,

500 μg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0104S/L v2.0

HindIII Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R0104LVIAL	HindIII	10176930	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10173660	Pass	
B6002SVIAL	NEBuffer™ r2.1	10154052	Pass	

Assay Name/Specification	Lot # 10176929
Blue-White Screening (Terminal Integrity)	Pass
A sample of Litmus28i vector linearized with a 10-fold excess of HindIII, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	
Ligation and Recutting (Terminal Integrity) After a 200-fold over-digestion of Lambda DNA with HindIII, >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 HindIII.	Pass
Protein Purity Assay (SDS-PAGE) HindIII is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
Exonuclease Activity (Radioactivity Release) A 50 μl reaction in NEBuffer™ r2.1 containing 1 μg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 200 units of HindIII incubated	Pass



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Assay Name/Specification	Lot # 10176929
for 4 hours at 37°C releases <0.1% of the total radioactivity.	
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of HindIII incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of HindIII 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
Functional Testing (15 minute Digest) A 50 μl reaction in NEBuffer™ r2.1 containing 1 μg of Lambda DNA and 1 μl of HindIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and a minimum of 60 units of HindIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	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 Sun \
Production Scientist
17 Jan 2023

Michael Tonello

Packaging Quality Control Inspector

18 Jan 2023

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