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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: 10204371
Expiration Date: 08/2025
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	10201063	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10207417	Pass	
B6002SVIAL	NEBuffer™ r2.1	10173667	Pass	

Assay Name/Specification	Lot # 10204371
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.	
Endonuclease Activity (Nicking)	Pass
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.	
Exonuclease Activity (Radioactivity Release)	Pass
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	
for 4 hours at 37°C releases <0.1% of the total radioactivity.	
Functional Testing (15 minute Digest)	Pass
A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and 1 µl of	



R0104L / Lot: 10204371

Page 1 of 2

Assay Name/Specification	Lot # 10204371
HindIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	
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
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
Protein Purity Assay (SDS-PAGE) HindIII 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 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

This product has been tested and shown to be in compliance with all specifications.

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YunJie Sun \ Production Scientist 06 Aug 2023 Josh Hersey

Packaging Quality Control Inspector

01 Dec 2023



R0104L / Lot: 10204371 Page 2 of 2