

www.neb.com info@neb.com



New England Biolabs Certificate of Analysis

Product Name: HindIII-HF®
Catalog Number: R3104L
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: 10249226
Expiration Date: 06/2026
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-R3104S/L/V v2.0

HindIII-HF® Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R3104LVIAL	HindIII-HF®	10245399	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10236423	Pass	
B6004SVIAL	rCutSmart™ Buffer	10243105	Pass	

Assay Name/Specification	Lot # 10249226
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of HindIII-HF® incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 200 units of HindIII-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Functional Testing (15 minute Digest) A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of Lambda DNA and 1 μl of HindIII-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
Ligation and Recutting (Terminal Integrity) After a 100-fold over-digestion of Lambda DNA with HindIII-HF®, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated	Pass



R3104L / Lot: 10249226

Page 1 of 2



Assay Name/Specification	Lot # 10249226
fragments, >95% can be recut with HindIII-HF®.	
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 200 units of HindIII-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) HindIII-HF® 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-HF® 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.

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.

Ana Egana Production Scientist 26 Jun 2024 Michael Tonello

Packaging Quality Control Inspector

26 Jun 2024



R3104L / Lot: 10249226

Page 2 of 2