

*be* INSPIRED *drive* DISCOVERY *stay* GENUINE

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	HindIII-HF®
Catalog Number:	R3104S
Concentration:	20,000 U/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
Packaging Lot Number:	10171791
Expiration Date:	06/2024
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3104SVIAL	HindIII-HF®	10152974	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10167588	Pass	
B6004SVIAL	rCutSmart™ Buffer	10165691	Pass	

Assay Name/Specification	Lot # 10171791
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart <sup>™</sup> 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
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart <sup>™</sup> 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
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] 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
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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.	Pass





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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.	
<b>Ligation and Recutting (Terminal Integrity)</b> 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 fragments, >95% can be recut with HindIII-HF®.	Pass
Protein Purity Assay (SDS-PAGE) HindIII-HF® is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart <sup>™</sup> 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

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.

Penghua Zhang Production Scientist 16 Jun 2022

Michae

Michael Tonello Packaging Quality Control Inspector 16 Nov 2022

