

## 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 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10167246  
**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			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3104SVIAL	HindIII-HF®	10152974	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10162784	Pass
B6004SVIAL	rCutSmart™ Buffer	10162783	Pass

Assay Name/Specification	Lot # 10167246
<p><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. 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.</p>	Pass
<p><b>Protein Purity Assay (SDS-PAGE)</b>            HindIII-HF® is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>Non-Specific DNase Activity (16 Hour)</b>            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.</p>	Pass
<p><b>Ligation and Recutting (Terminal Integrity)</b></p>	Pass

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<p>After a 100-fold over-digestion of Lambda DNA with HindIII-HF<sup>®</sup>, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with HindIII-HF<sup>®</sup>.</p>	
<p><b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and 1 µl of HindIII-HF<sup>®</sup> incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Exonuclease Activity (Radioactivity Release)</b> 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<sup>®</sup> incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking)</b> 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<sup>®</sup> incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>

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](http://www.neb.com/trademarks) for additional information.



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