

## New England Biolabs Certificate of Analysis

**Product Name:** HindIII  
**Catalog Number:** R0104L  
**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 NEBuffer r2.1 in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10152728  
**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-R0104S/L v2.0

| HindIII Component List |                              |            |                      |
|------------------------|------------------------------|------------|----------------------|
| NEB Part Number        | Component Description        | Lot Number | Individual QC Result |
| R0104LVIAL             | HindIII                      | 10152724   | Pass                 |
| B7024AVIAL             | Gel Loading Dye, Purple (6X) | 10152092   | Pass                 |
| B6002SVIAL             | NEBuffer™ r2.1               | 10151373   | Pass                 |

| Assay Name/Specification   | Lot # 10152728 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>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.           | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>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           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>HindIII is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.  | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>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%   | Pass           |

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|--|----------------|
| conversion to the nicked form as determined by agarose gel electrophoresis.  |                |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>A 50 µl reaction in NEBuffer™ r2.1 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 incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>   | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>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.</p> | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>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.</p>  | <b>Pass</b>    |
| <p><b>Functional Testing (15 minute Digest)</b><br/>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.</p>  | <b>Pass</b>    |

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

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12 Jul 2022

  
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