

## 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:** 10179269  
**Expiration Date:** 12/2024  
**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

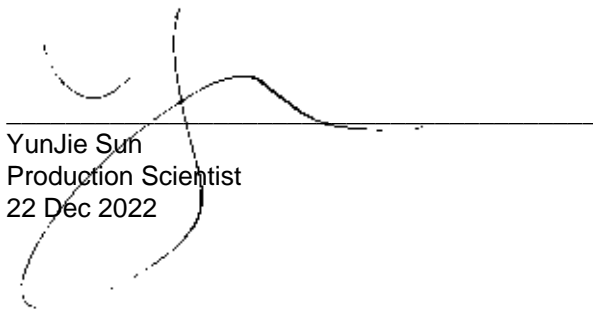
| HindIII-HF® Component List |                              |            |                      |
|----------------------------|------------------------------|------------|----------------------|
| NEB Part Number            | Component Description        | Lot Number | Individual QC Result |
| R3104SVIAL                 | HindIII-HF®                  | 10174623   | Pass                 |
| B7024AVIAL                 | Gel Loading Dye, Purple (6X) | 10182168   | Pass                 |
| B6004SVIAL                 | rCutSmart™ Buffer            | 10182167   | Pass                 |

| Assay Name/Specification   | Lot # 10179269 |
|--|----------------|
| <b>Endonuclease Activity (Nicking)</b><br>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           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in rCutSmart™ 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>Functional Testing (15 minute Digest)</b><br>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           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>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           |

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|--|----------------|
| <p>fragments, &gt;95% can be recut with HindIII-HF®.</p> <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>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>                               | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>HindIII-HF® is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>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> | <b>Pass</b>    |

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

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