

## New England Biolabs Certificate of Analysis

**Product Name:** EcoRI-HF<sup>®</sup>  
**Catalog Number:** R3101M  
**Concentration:** 100,000 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:** 10091891  
**Expiration Date:** 12/2022  
**Storage Temperature:** -20°C  
**Storage Conditions:** 300 mM NaCl, 10 mM KPO<sub>4</sub>, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % TritonX-100, 200 µg/ml BSA, (pH 7.0 @ 25°C)  
**Specification Version:** PS-R3101T/M v2.0

| EcoRI-HF <sup>®</sup> Component List |                              |            |                      |
|--------------------------------------|------------------------------|------------|----------------------|
| NEB Part Number                      | Component Description        | Lot Number | Individual QC Result |
| R3101M VIAL                          | EcoRI-HF <sup>®</sup>        | 10091885   | Pass                 |
| B7204S VIAL                          | CutSmart <sup>®</sup> Buffer | 10093127   | Pass                 |
| B7024A VIAL                          | Gel Loading Dye, Purple (6X) | 10091034   | Pass                 |

| Assay Name/Specification   | Lot # 10091891 |
|--|----------------|
| <b>Protein Purity Assay (SDS-PAGE)</b><br>EcoRI-HF <sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.  | Pass           |
| <b>Non-Specific DNase Activity (16 Hour)</b><br>A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 100 Units of EcoRI-HF <sup>™</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 20-fold over-digestion of Lambda DNA with EcoRI-HF <sup>™</sup> , >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 EcoRI-HF <sup>™</sup> .                                      | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in CutSmart <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 EcoRI-HF <sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.    | Pass           |

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|--|----------------|
| <p><b>Endonuclease Activity (Nicking)</b><br/>A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of EcoRI-HF™ incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p> | <b>Pass</b>    |
| <p><b>Blue-White Screening (Terminal Integrity)</b><br/>A sample of pUC19 vector linearized with a 10-fold excess of EcoRI-HF™, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</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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24 Feb 2021



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24 Feb 2021