

## New England Biolabs Certificate of Analysis

**Product Name:** EcoRI-HF®  
**Catalog Number:** R3101S  
**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:** 10073657  
**Expiration Date:** 12/2021  
**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-R3101S/L v2.0

| EcoRI-HF® Component List |                              |            |                      |
|--------------------------|------------------------------|------------|----------------------|
| NEB Part Number          | Component Description        | Lot Number | Individual QC Result |
| R3101SVIAL               | EcoRI-HF®                    | 10061390   | Pass                 |
| B7204SVIAL               | CutSmart® Buffer             | 10074633   | Pass                 |
| B7024SVIAL               | Gel Loading Dye, Purple (6X) | 10074634   | Pass                 |

| Assay Name/Specification   | Lot # 10073657 |
|--|----------------|
| <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 <1% white colonies.   | Pass           |
| <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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.            | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in CutSmart™ 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™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 20-fold over-digestion of Lambda DNA with EcoRI-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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|--|----------------|
| fragments, >95% can be recut with EcoRI-HF™.   |                |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 Units of EcoRI-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/>EcoRI-HF™ is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |

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



Penghua Zhang  
Production Scientist  
26 Jun 2020



Michael Tonello  
Packaging Quality Control Inspector  
26 Jun 2020