

## New England Biolabs Certificate of Analysis

**Product Name:** EcoRI-HF®  
**Catalog Number:** R3101T  
**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:** 10173152  
**Expiration Date:** 12/2024  
**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® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3101TVIAL	EcoRI-HF®	10173148	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10168649	Pass
B6004SVIAL	rCutSmart™ Buffer	10173161	Pass

Assay Name/Specification	Lot # 10173152
<b>Protein Purity Assay (SDS-PAGE)</b> EcoRI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> 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.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> 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 fragments, >95% can be recut with EcoRI-HF™.	Pass
<b>Blue-White Screening (Terminal Integrity)</b> 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

Assay Name/Specification	Lot # 10173152
<p><b>Endonuclease Activity (Nicking)</b> 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>Exonuclease Activity (Radioactivity Release)</b> 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 &lt;0.1% of the total radioactivity.</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.




---

YunJie Sun  
Production Scientist  
14 Dec 2022




---

Michael Tonello  
Packaging Quality Control Inspector  
15 Dec 2022