

## 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 rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10211302  
**Expiration Date:** 10/2025  
**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 % Triton X-100, 200 µg/ml rAlbumin, (pH 7.0 @ 25°C)  
**Specification Version:** PS-R3101S/L v3.0

EcoRI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3101SVIAL	EcoRI-HF®	10211229	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10202501	Pass
B6004SVIAL	rCutSmart™ Buffer	10207415	Pass

Assay Name/Specification	Lot # 10211302
<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
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in rCutSmart™ 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> 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 EcoRI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of	Pass

Assay Name/Specification	Lot # 10211302
<p>EcoRI-HF<sup>®</sup> incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of Lambda DNA with EcoRI-HF<sup>®</sup>, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with EcoRI-HF<sup>®</sup>.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in rCutSmart<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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> EcoRI-HF<sup>®</sup> is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of EcoRI-HF<sup>®</sup> is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 20 units of EcoRI-HF<sup>®</sup> is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> 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.

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  
25 Oct 2023

*Michael Tonello*

---

Michael Tonello  
Packaging Quality Control Inspector  
13 Nov 2023