

## New England Biolabs Certificate of Analysis

**Product Name:** EcoRI-HF<sup>®</sup>  
**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:** 10200663  
**Expiration Date:** 01/2025  
**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 % TritonX-100, 200 µg/ml BSA, (pH 7.0 @ 25°C)  
**Specification Version:** PS-R3101S/L v2.0

EcoRI-HF <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3101SVIAL	EcoRI-HF <sup>®</sup>	10176738	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10193044	Pass
B6004SVIAL	rCutSmart <sup>™</sup> Buffer	10196036	Pass

Assay Name/Specification	Lot # 10200663
<b>Blue-White Screening (Terminal Integrity)</b> A sample of pUC19 vector linearized with a 10-fold excess of EcoRI-HF <sup>™</sup> , 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 CutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of EcoRI-HF <sup>™</sup> 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 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
<b>Ligation and Recutting (Terminal Integrity)</b> 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	Pass

