

## New England Biolabs Certificate of Analysis

**Product Name:** EcoRI-HF™  
**Catalog #:** R3101S/L  
**Concentration:** 20,000 units/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.  
**Lot #:** 0081301  
**Assay Date:** 01/2013  
**Expiration Date:** 01/2015  
**Storage Temp:** -20 °C  
**Storage Conditions:** 300 mM NaCl, 10 mM KPO4 (pH 7.5), 10 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton X-100, 200 µg/ml BSA  
**Specification Version:** PS-R3101S/L v1.0  
**Effective Date:** 17 Apr 2013

Assay Name/Specification (minimum release criteria)	Lot #0081301
<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 <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	<b>Pass</b>
<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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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] <i>E. coli</i> DNA and a minimum of 200 units of EcoRI-HF™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<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™.	<b>Pass</b>
<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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - EcoRI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

M. W. Southworth

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Authorized by  
Maurice Southworth  
17 Apr 2013

C. Madinger

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Inspected by  
Casey Madinger  
17 Apr 2013

