

## New England Biolabs Certificate of Analysis

*Product Name:* ApoI-HF  
*Catalog #:* R3566S/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 degrees C in a total reaction volume of 50 µL  
*Lot #:* 0021609  
*Assay Date:* 09/2016  
*Expiration Date:* 09/2018  
*Storage Temp:* -20°C  
*Storage Conditions:* 200 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 200 µg/ml BSA, (pH 7.4 @ 25°C)  
*Specification Version:* PS-R3566S/L v1.0  
*Effective Date:* 06 Jan 2016

Assay Name/Specification (minimum release criteria)	Lot #0021609
<b>Blue-White Screening (Terminal Integrity)</b> - A sample of pUC19 vector linearized with a 10-fold excess of ApoI-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>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 100 units of ApoI-HF incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (15 minute Digest)</b> - A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda DNA and 1 µl of ApoI-HF incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 20-fold over-digestion of Lambda DNA with ApoI-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 ApoI-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 ApoI-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> - ApoI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
06 Jan 2016



Inspected by  
Anthony Francis  
09 Sep 2016

