

## New England Biolabs Certificate of Analysis

**Product Name:** Apol-HF<sup>®</sup>  
**Catalog Number:** R3566L  
**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 degrees C in a total reaction volume of 50 µL  
**Packaging Lot Number:** 10070691  
**Expiration Date:** 01/2022  
**Storage Temperature:** -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

Apol-HF <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3566LVIAL	Apol-HF <sup>®</sup>	10063404	Pass
B7204SVIAL	CutSmart <sup>®</sup> Buffer	10071078	Pass
B7024SVIAL	Gel Loading Dye, Purple (6X)	10065747	Pass

Assay Name/Specification	Lot # 10070691
<b>Blue-White Screening (Terminal Integrity)</b> A sample of pUC19 vector linearized with a 10-fold excess of Apol-HF, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	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 100 units of Apol-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 CutSmart <sup>®</sup> Buffer containing 1 µg of Lambda DNA and 1 µl of Apol-HF incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of Lambda DNA with Apol-HF, >95% of the DNA fragments	Pass

Assay Name/Specification	Lot # 10070691
<p>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with Apol-HF.</p>	
<p><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 Apol-HF 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> Apol-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.




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Penghua Zhang  
Production Scientist  
05 May 2020




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Jay Minichiello  
Packaging Quality Control Inspector  
05 May 2020