

New England Biolabs Product Specification

Product Name:	<i>ApoI-HF</i>
Catalog #:	<i>R3566S/L</i>
Concentration:	<i>20,000 units/ml</i>
Unit Definition:	<i>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</i>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20°C</i>
Storage Conditions:	<i>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)</i>
Specification Version:	<i>PS-R3566S/L v1.0</i>
Effective Date:	<i>06 Jan 2016</i>

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of ApoI-HF, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 100 units of ApoI-HF incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - 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.

Ligation and Recutting (Terminal Integrity) - 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.

Non-Specific DNase Activity (16 Hour) - 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.

Protein Purity Assay (SDS-PAGE) - ApoI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

* 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.



Date 06 Jan 2016

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Director of Quality Control

