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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 #:	*0021703*
Assay Date:	*03/2017*
Expiration Date:	*3/2019*
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 #0021703**
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.	**Pass**
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *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.	**Pass**
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.	**Pass**
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.	**Pass**
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.	**Pass**
Protein Purity Assay (SDS-PAGE) - ApoI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

Authorized by
Derek Robinson
06 Jan 2016

Inspected by
Anthony Francis
15 Mar 2017

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices