NEW ENGLAND BioLabs Inc.

240 County Road
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New England Biolabs
Certificate of Analysis

Product Name:	*NheI-HF™*
Catalog #:	*R3131M*
Concentration:	*100,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA (HindIII digest) in 1 hour at 37°C in a total reaction volume of 50 µl.*
Lot #:	*0021803*
Assay Date:	*03/2018*
Expiration Date:	*3/2020*
Storage Temp:	*-20°C*
Storage Conditions:	*250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton X-100, 200 µg/ml BSA*
Specification Version:	*PS-R3131M v1.0*
Effective Date:	*17 Apr 2013*

Assay Name/Specification (minimum release criteria)	**Lot #0021803**
Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of NheI-HF™, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.	**Pass**
Endonuclease Activity (Nicking) - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of NheI-HF™ incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**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 300 units of NheI-HF™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Ligation and Recutting (Terminal Integrity) - After a 100-fold over-digestion of Lambda HindIII DNA with NheI-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 NheI-HF™.	**Pass**
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda HindIII DNA and a minimum of 200 Units of NheI-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**

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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

Authorized by
Maurice Southworth
17 Apr 2013

Stephanie Cornelio

Inspected by
Stephanie Cornelio
21 Feb 2018

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