NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*NcoI-HF®*
Catalog #:	*R3193M*
Concentration:	*100,000 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°C in a total reaction volume of 50 µl.*
Shelf Life:	*24 months*
Storage Temp:	*-20 °C*
Storage Conditions:	*200 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA*
Specification Version:	*PS-R3193M v1.0*
Effective Date:	*10 Jun 2013*

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI-HF™, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

Endonuclease Activity (Nicking) - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 Units of NcoI-HF™ incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

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 NcoI-HF™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of Lambda DNA with NcoI-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 NcoI-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 NcoI-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) - NcoI-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.*

Derek Robinson
Director of Quality Control

Date 10 Jun 2013

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