

## New England Biolabs Product Specification

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|-------------------------------|---|
| <b>Product Name:</b>          | <i>NheI-HF</i> <sup>®</sup>   |
| <b>Catalog #:</b>             | R3131S/L  |
| <b>Concentration:</b>         | 20,000 units/ml   |
| <b>Unit Definition:</b>       | One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA ( <i>HindIII</i> digest) in 1 hour at 37°C in a total reaction volume of 50 µl. |
| <b>Shelf Life:</b>            | 24 months   |
| <b>Storage Temp:</b>          | -20 °C  |
| <b>Storage Conditions:</b>    | 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  |
| <b>Specification Version:</b> | PS-R3131S/L v1.0  |
| <b>Effective Date:</b>        | 24 Apr 2013   |

### Assay Name/Specification (minimum release criteria)

**Blue-White Screening (Terminal Integrity)** - A sample of LITMUS28i vector linearized with a 10-fold excess of *NheI-HF*<sup>™</sup>, 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<sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of *NheI-HF*<sup>™</sup> 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<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 300 units of *NheI-HF*<sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Ligation and Recutting (Terminal Integrity)** - After a 100-fold over-digestion of Lambda *HindIII* DNA with *NheI-HF*<sup>™</sup>, >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*<sup>™</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in CutSmart<sup>™</sup> Buffer containing 1 µg of Lambda *HindIII* DNA and a minimum of 200 Units of *NheI-HF*<sup>™</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

\* 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 24 Apr 2013

Derek Robinson

Director of Quality Control

