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## New England Biolabs Product Specification

Product Name:	HindIII-HF®
Catalog #:	R3104S/L
Concentration:	20,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
Shelf Life:	24 months
Storage Temp:	-20 °C
Storage Conditions:	300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 μg/ml BSA
Specification Version:	PS-R3104S/L v1.0
Effective Date:	10 May 2013

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in CutSmart<sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 Units of HindIII-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  $\mu$ l reaction in CutSmart<sup>TM</sup> Buffer containing 1  $\mu$ g of a mixture of single and doublestranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 200 units of HindIII-HF<sup>TM</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 DNA with HindIII-HF<sup>TM</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 HindIII-HF<sup>TM</sup>.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 200 Units of HindIII-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.

Protein Purity Assay (SDS-PAGE) - HindIII-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 May 2013