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## New England Biolabs Product Specification

Product Name: Hinfl

Catalog #: R0155T/M
Concentration: 50,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C

in a total reaction of 50  $\mu$ l.

Shelf Life: 24 months
Storage Temp: -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0155T/M v2.0

Effective Date: 02 Mar 2023

## Assay Name/Specification (minimum release criteria)

Exonuclease Activity (Radioactivity Release) - A 50  $\mu$ l reaction in rCutSmart<sup>TM</sup> Buffer containing 1  $\mu$ g of a mixture of single and double-stranded [  $^3$ H] *E. coli* DNA and a minimum of 100 units of Hinfl incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 μl reaction in rCutSmart<sup>TM</sup> Buffer containing 1 μg of Lambda DNA and 1 μl of Hinfl incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

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

Non-Specific DNase Activity (16 Hour) - A 50 μl reaction in rCutSmart<sup>TM</sup> Buffer containing 1 μg of Lambda DNA and a minimum of 100 units of HinfI 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) - Hinfl is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 10 units of Hinf1 is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is  $\leq 1$  *E. coli* genome.

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Date 02 Mar 2023

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