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## New England Biolabs Product Specification

Product Name: EcoRV-HF®

Catalog #: R3195S/L/V

Concentration: 20,000 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-R3195S/L v1.0
Effective Date: 05/06/2013

## Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of EcoRV-HF<sup>TM</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>TM</sup> Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 20 Units of EcoRV-HF<sup>TM</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 μl reaction in CutSmart<sup>TM</sup> Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup>H] *E. coli* DNA and a minimum of 100 units of EcoRV-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 10-fold over-digestion of Lambda DNA with EcoRV-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 EcoRV-HF<sup>TM</sup>.

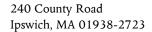
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart<sup>TM</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 100 Units of EcoRV-HF<sup>TM</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

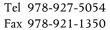
Protein Purity Assay (SDS-PAGE) - EcoRV-HFTM is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.











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Date 05/06/2013

Derek Robinson Quality Approver





