New England Biolabs
Product Specification

Product Name: EcoRI-HF®
Catalog #: R3101T/M
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: 300 mM NaCl, 10 mM KPO4, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % TritonX-100, 200 µg/ml BSA, (pH 7.0 @ 25°C)
Specification Version: PS-R3101T/M v2.0
Effective Date: 16 May 2016

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of EcoRI-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 100 Units of EcoRI-HF™ incubated for 4 hours at 37ºC 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 [³H] E. coli DNA and a minimum of 200 units of EcoRI-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 EcoRI-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 EcoRI-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 EcoRI-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) - EcoRI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.
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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (H 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 16 May 2016