

New England Biolabs Product Specification

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| Product Name: | EcoRI-HF [®] |
| Catalog #: | R3101S/L |
| 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 rCutSmart Buffer 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 KPO ₄ , 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % Triton X-100, 200 µg/ml rAlbumin, (pH 7.0 @ 25°C) |
| Specification Version: | PS-R3101S/L v3.0 |
| Effective Date: | 07 Nov 2023 |

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 rCutSmart[™] Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of EcoRI-HF[®] 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 rCutSmart[™] 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.

Functional Testing (15 minute Digest) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of Lambda DNA and 1 µl of EcoRI-HF[®] 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 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 rCutSmart[™] 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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qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of EcoRI-HF[®] 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 ≤ 1 *E. coli* genome.

RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of EcoRI-HF[®] is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

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Date 07 Nov 2023

Nancy Considine
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