

New England Biolabs Product Specification

Product Name:	<i>EcoRI</i>
Catalog #:	R0101S/L/E
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 NEBuffer <i>EcoRI/SspI</i> 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 7.5 @ 25°C)
Specification Version:	PS-R0101S/L/E v4.0
Effective Date:	19 Feb 2024

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of *EcoRI*, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

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

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

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer *EcoRI/SspI* containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 200 units of *EcoRI* incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 µl reaction in 1X NEBuffer *EcoRI/SspI* containing 1 µg of Lambda DNA and 1 µl of *EcoRI* incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer *EcoRI/SspI* containing 1 µg of Lambda DNA and a minimum of 100 units of *EcoRI* incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of *EcoRI* 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.





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Date 19 Feb 2024

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Quality Approver

