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New England Biolabs Certificate of Analysis

Product Name: AvrII

Catalog Number: R0174L

Concentration: 5,000 U/mI

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg

of Lambda DNA (HindIII digest) in rCutSmart Buffer in 1 hour at 37°C

in a total reaction volume of 50 μl.

Packaging Lot Number: 10165129
Expiration Date: 06/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol,

500 μg/ml rAlbumin (pH 7.4 @25°C)

Specification Version: PS-R0174S/L/V v2.0

AvrII Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R0174LVIAL	AvrII	10152551	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10161525	Pass	
B6004SVIAL	rCutSmart™ Buffer	10162781	Pass	

Assay Name/Specification	Lot # 10165129
Protein Purity Assay (SDS-PAGE)	Pass
AvrII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	
Blue-White Screening (Terminal Integrity)	Pass
A sample of Litmus28i vector linearized with a 10-fold excess of AvrII, religated	
and transformed into an E. coli strain expressing the LacZ beta fragment gene	
results in <1% white colonies.	
Ligation and Recutting (Terminal Integrity)	Pass
After a 50-fold over-digestion of Lambda HindIII DNA with AvrII, >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 AvrII.	
qPCR DNA Contamination (E. coli Genomic)	Pass
A minimum of 5 units of AvrII 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	



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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.	
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 50 units of AvrII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Functional Testing (15 minute Digest) A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of Lambda-HindIII DNA and 1 μl of AvrII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
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 50 units of AvrII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of AvrII incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass

This product has been tested and shown to be in compliance with all specifications.

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Penghaa Zhang Production Scientist

06 Jul 2022

Josh Hersey

Packaging Quality Control Inspector

07 Oct 2022



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