

New England Biolabs Certificate of Analysis

Product Name: *AvrII*
Catalog Number: *R0174L*
Concentration: *5,000 U/ml*
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: *10136969*
Expiration Date: *11/2023*
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	10128047	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10130600	Pass
B6004SVIAL	rCutSmart™ Buffer	10131975	Pass

Assay Name/Specification	Lot # 10136969
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
Protein Purity Assay (SDS-PAGE) AvrII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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 [³ H] E. coli DNA and a minimum of 50 units of AvrII incubated for 4	Pass

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hours at 37°C releases <0.1% of the total radioactivity.	
<p>Blue-White Screening (Terminal Integrity) 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.</p>	Pass
<p>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.</p>	Pass
<p>qPCR DNA Contamination (E. coli Genomic) 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 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.</p>	Pass
<p>Ligation and Recutting (Terminal Integrity) 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.</p>	Pass

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

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07 Feb 2022



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07 Feb 2022