

New England Biolabs Certificate of Analysis

Product Name: *AvrII*
Catalog Number: *R0174S*
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: *10128049*
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 |
| R0174SVIAL | AvrII | 10128048 | Pass |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10121396 | Pass |
| B6004SVIAL | rCutSmart™ Buffer | 10127378 | Pass |

| Assay Name/Specification | Lot # 10128049 |
|--|----------------|
| <p>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.</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 |

| Assay Name/Specification | Lot # 10128049 |
|--|----------------|
| <p>Protein Purity Assay (SDS-PAGE) AvrII is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</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 |
| <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>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.</p> | Pass |
| <p>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.</p> | Pass |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



Penghua Zhang
Production Scientist
01 Dec 2021



Michael Tonello
Packaging Quality Control Inspector
01 Dec 2021