

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

| Product Name:          | Avrll   |
|------------------------|---|
| Catalog Number:        | R0174L  |
| Concentration:         | 5,000 U/ml  |
| Unit Definition:       | One unit is defined as the amount of enzyme required to digest 1 μg<br>of Lambda DNA (HindIII digest) in rCutSmart Buffer in 1 hour at 37°C<br>in a total reaction volume of 50 μl. |
| Packaging Lot Number:  | 10227036  |
| Expiration Date:       | 09/2025   |
| Storage Temperature:   | -20°C   |
| Storage Conditions:    | 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol,<br>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           | Avrll                        | 10206311   | Pass                 |  |
| B7024AVIAL           | Gel Loading Dye, Purple (6X) | 10221468   | Pass                 |  |
| B6004SVIAL           | rCutSmart™ Buffer            | 10224841   | Pass                 |  |

| Assay Name/Specification   | Lot # 10227036 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>A sample of Litmus28i vector linearized with a 10-fold excess of AvrII, religated<br>and transformed into an E. coli strain expressing the LacZ beta fragment gene<br>results in <1% white colonies.   | Pass           |
| Endonuclease Activity (Nicking)<br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and<br>a minimum of 50 units of AvrII incubated for 4 hours at 37°C results in <10%<br>conversion to the nicked form as determined by agarose gel electrophoresis.           | Pass           |
| Exonuclease Activity (Radioactivity Release)<br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of a mixture of single and<br>double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 50 units of AvrII incubated for 4<br>hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| Functional Testing (15 minute Digest)<br>A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of Lambda-HindIII DNA and 1 μl  | Pass           |





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| of AvrII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.  |                |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 50-fold over-digestion of Lambda HindIII DNA with AvrII, >95% of the DNA<br>fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated<br>fragments, >95% can be recut with AvrII.  | Pass           |
| Non-Specific DNase Activity (16 Hour)<br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of Lambda-HindIII DNA and a<br>minimum of 50 units of AvrII incubated for 16 hours at 37°C results in a DNA pattern<br>free of detectable nuclease degradation as determined by agarose gel<br>electrophoresis.   | Pass           |
| Protein Purity Assay (SDS-PAGE)<br>AvrII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue<br>detection.   | Pass           |
| <b>qPCR DNA Contamination (E. coli Genomic)</b><br>A minimum of 5 units of AvrII is screened for the presence of E. coli genomic DNA<br>using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results<br>are quantified using a standard curve generated from purified E. coli genomic DNA.<br>The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome. | Pass           |

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

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YunJie Sun Production Scientist 19 Sep 2023

Michae

Michael Tonello Packaging Quality Control Inspector 15 Feb 2024

