

## 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:** *10264803*  
**Expiration Date:** *05/2026*  
**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*

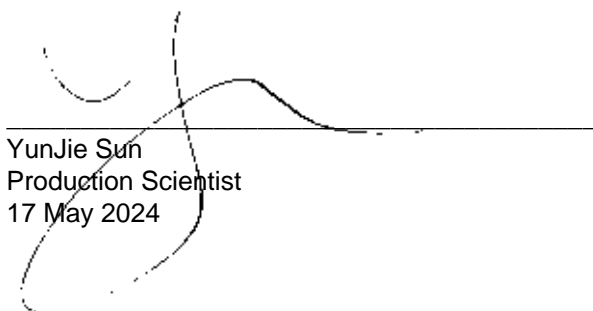
| AvrII Component List |                              |            |                      |
|----------------------|------------------------------|------------|----------------------|
| NEB Part Number      | Component Description        | Lot Number | Individual QC Result |
| R0174LVIAL           | AvrII                        | 10241582   | Pass                 |
| B7024AVIAL           | Gel Loading Dye, Purple (6X) | 10250204   | Pass                 |
| B6004SVIAL           | rCutSmart™ Buffer            | 10256011   | Pass                 |

| Assay Name/Specification  | Lot # 10264803 |
|---|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>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.  | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>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           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] 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           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII DNA and 1 µl  | Pass           |

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|---|----------------|
| <p>of AvrII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>  |                |
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>After a 50-fold over-digestion of Lambda HindIII DNA with AvrII, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with AvrII.</p>   | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>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>  | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>AvrII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>  | <b>Pass</b>    |
| <p><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 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> | <b>Pass</b>    |

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

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YunJie Sun  
Production Scientist  
17 May 2024



Michael Tonello  
Packaging Quality Control Inspector  
14 Nov 2024