

## New England Biolabs Product Specification

|                               |  |
|-------------------------------|--|
| <i>Product Name:</i>          | <i>AvrII</i>   |
| <i>Catalog #:</i>             | <i>R0174S/L/V</i>  |
| <i>Concentration:</i>         | <i>5,000 units/ml</i>  |
| <i>Unit Definition:</i>       | <i>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.</i> |
| <i>Shelf Life:</i>            | <i>24 months</i>   |
| <i>Storage Temp:</i>          | <i>-20°C</i>   |
| <i>Storage Conditions:</i>    | <i>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)</i>   |
| <i>Specification Version:</i> | <i>PS-R0174S/L/V v2.0</i>  |
| <i>Effective Date:</i>        | <i>23 Nov 2021</i>   |

### Assay Name/Specification (minimum release criteria)

**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.

**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.

**Protein Purity Assay (SDS-PAGE)** - AvrII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**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.

**Exonuclease Activity (Radioactivity Release)** - 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.

**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.

**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.



---

## New England Biolabs Product Specification

| Assay Name/Specification (minimum release criteria) |
|---|
|---|

|   |
|---|
| qPCR DNA Contamination ( <i>E. coli</i> Genomic) - A minimum of 5 units of AvrII is screened for the presence of <i>E. coli</i> genomic DNA using SYBR <sup>®</sup> Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is $\leq 1$ <i>E. coli</i> genome. |
|---|

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



Date 23 Nov 2021

---

Derek Robinson  
Director, Quality Control

