

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>AflIII</i>
<i>Catalog #:</i>	<i>R0541S/L</i>
<i>Concentration:</i>	<i>10,000 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 in NEBuffer™ r3.1 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, 500 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-R0541S/L v2.0</i>
<i>Effective Date:</i>	<i>03 Mar 2022</i>

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

**Exonuclease Activity (Radioactivity Release)** - A 50 µl reaction in NEBuffer™ r3.1 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 AflIII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (15 minute Digest)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of AflIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

**Ligation and Recutting (Terminal Integrity)** - After a 5-fold over-digestion of Lambda DNA with AflIII, >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 AflIII.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 30 units of AflIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 10 units of AflIII 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.

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