

## New England Biolabs Certificate of Analysis

**Product Name:** AfIII

**Catalog Number:** R0541L

**Concentration:** 10,000 U/ml

**Unit Definition:** 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.

**Packaging Lot Number:** 10181582

**Expiration Date:** 03/2025

**Storage Temperature:** -20°C

**Storage Conditions:** 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)

**Specification Version:** PS-R0541S/L v2.0

AfIII Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0541LVIAL	AfIII	10181581	Pass
B6003SVIAL	NEBuffer™ r3.1	10182163	Pass

Assay Name/Specification	Lot # 10181582
<p><b>Exonuclease Activity (Radioactivity Release)</b> 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 AfIII incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of AfIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 5-fold over-digestion of Lambda DNA with AfIII, &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 AfIII.</p>	Pass
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 30 units of AfIII incubated for 16 hours at 37°C results in a DNA pattern free of</p>	Pass

Assay Name/Specification	Lot # 10181582
detectable nuclease degradation as determined by agarose gel electrophoresis.	
<b>Protein Purity Assay (SDS-PAGE)</b> AflIII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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.	<b>Pass</b>


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

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14 Mar 2023




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07 Apr 2023