

## 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:** 10158865

**Expiration Date:** 08/2024

**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	10158863	Pass
B6003SVIAL	NEBuffer™ r3.1	10146825	Pass

Assay Name/Specification	Lot # 10158865
<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>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>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

