

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: 10137184

Expiration Date: 01/2024

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	10137159	Pass
B6003SVIAL	NEBuffer™ r3.1	10126635	Pass

Assay Name/Specification	Lot # 10137184
<p>qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of AfIII 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>	Pass
<p>Protein Purity Assay (SDS-PAGE) AfIII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>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 AfIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	Pass
<p>Functional Testing (15 minute Digest) 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</p>	Pass

Assay Name/Specification	Lot # 10137184
agarose gel electrophoresis.	
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [³ 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.	Pass
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.	Pass

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

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Production Scientist
04 Mar 2022



Mary Neal
Packaging Quality Control Inspector
04 Mar 2022