

*be* INSPIRED *drive* DISCOVERY *stay* GENUINE

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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

AfIIII 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
<b>Ligation and Recutting (Terminal Integrity)</b> After a 5-fold over-digestion of Lambda DNA with AfIIII, >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 AfIIII.	Pass
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 AfIIII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Functional Testing (15 minute Digest) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of AfIIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
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 AfIIII incubated for 16 hours at 37°C results in a DNA pattern free of	Pass





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Assay Name/Specification	Lot # 10158865
detectable nuclease degradation as determined by agarose gel electrophoresis.	
qPCR DNA Contamination (E. coli Genomic)	Pass
A minimum of 10 units of AfIIII 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 $\leq$ 1 E. coli genome.	
Protein Purity Assay (SDS-PAGE)	Pass
AfIIII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	

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

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

Penghua Zhang

Penghua Zhang Production Scientist 22 Aug 2022

Erin Varney

Packaging Quality Control Inspector 22 Aug 2022

