

## New England Biolabs Certificate of Analysis

**Product Name:** AfIII  
**Catalog Number:** R0520L  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of PhiX174 RF I DNA in rCutSmart™ Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10239246  
**Expiration Date:** 02/2026  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0520S/L v2.0

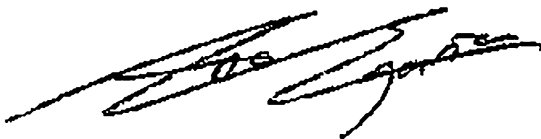
AfIII Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0520LVIAL	AfIII	10230764	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10234872	Pass
B6004SVIAL	rCutSmart™ Buffer	10233338	Pass

Assay Name/Specification	Lot # 10239246
<b>Blue-White Screening (Terminal Integrity)</b> A sample of Litmus28 vector linearized with a 10-fold excess of AfIII, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	<b>Pass</b>
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pBR322 DNA and a minimum of 100 units of AfIII incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of AfIII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of PhiX174 RF I DNA and 1 µl	<b>Pass</b>

Assay Name/Specification	Lot # 10239246
<p>of AfIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 5-fold over-digestion of PhiX174 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>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of PhiX174 RF I DNA and a minimum of 200 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>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> AfIII is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 20 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>	<b>Pass</b>

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

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08 Apr 2024



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