

## New England Biolabs Certificate of Analysis

**Product Name:** AfIII  
**Catalog Number:** R0520S  
**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 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10091829  
**Expiration Date:** 09/2022  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R0520S/L v1.0

AfIII Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0520SVIAL	AfIII	10082432	Pass
B7204SVIAL	CutSmart® Buffer	10089407	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10089392	Pass

Assay Name/Specification	Lot # 10091829
<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.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in CutSmart™ 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.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart™ 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.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 5-fold over-digestion of PhiX174 DNA with AfIII, >95% of the DNA fragments	Pass

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<p>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with AflIII.</p> <p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of PhiX174 DNA and a minimum of 200 Units of AflIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<p><b>Pass</b></p>

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

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09 Dec 2020




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