

## 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 rCutSmart™ Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10208336  
**Expiration Date:** 08/2025  
**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

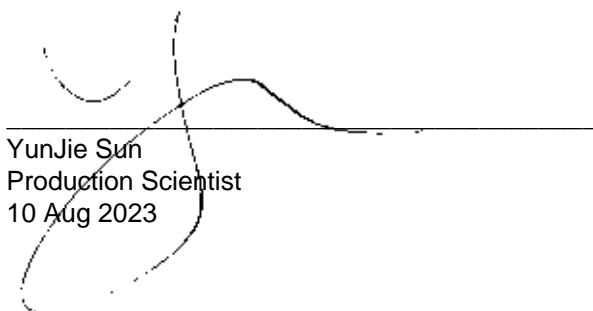
| AfIII Component List |                              |            |                      |
|----------------------|------------------------------|------------|----------------------|
| NEB Part Number      | Component Description        | Lot Number | Individual QC Result |
| R0520SVIAL           | AfIII                        | 10201746   | Pass                 |
| B7024AVIAL           | Gel Loading Dye, Purple (6X) | 10202499   | Pass                 |
| B6004SVIAL           | rCutSmart™ Buffer            | 10202504   | Pass                 |

| Assay Name/Specification   | Lot # 10208336 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>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><br>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.            | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>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. | Pass           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of PhiX174 RF I DNA and 1 µl   | Pass           |

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|--|----------------|
| <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><br/>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><br/>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><br/>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><br/>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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