

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 1 hour at 37°C in a total reaction volume of 50 µl.
Lot Number: 10028175
Expiration Date: 11/2020
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 |
| R0520LVIAL | AfIII | 10028174 | Pass |
| B7204SVIAL | CutSmart® Buffer | 10021119 | Pass |
| B7024SVIAL | Gel Loading Dye, Purple (6X) | 10018415 | Pass |

| Assay Name/Specification | Lot # 10028175 |
|---|----------------|
| Blue-White Screening (Terminal Integrity) 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 |
| Endonuclease Activity (Nicking) 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 |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ 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 |
| Ligation and Recutting (Terminal Integrity) 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, >95% can be recut with AflIII.</p> <p>Non-Specific DNase Activity (16 Hour) 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>Pass</p> |

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



Tony Spear-Alfonso
Production Scientist
07 Sep 2018



Michael Tonello
Packaging Quality Control Inspector
12 Nov 2018