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## New England Biolabs Certificate of Analysis

Product Name: AflII

R0520S/L Catalog #: Concentration: 20,000 units/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  $\mu$ l.

Lot #: 0421302 02/2013 Assay Date: 02/2015 Expiration Date: Storage Temp: -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

PS-R0520S/L v1.0 Specification Version: Effective Date: 12 Jun 2013

| Assay Name/Specification (minimum release criteria)   | Lot #0421302 |
|---|--------------|
| <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 <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.   | Pass         |
| Endonuclease Activity (Nicking) - A 50 μl reaction in CutSmart <sup>TM</sup> 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 <sup>TM</sup> Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> 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 can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with AfIII.  | Pass         |
| Non-Specific DNase Activity (16 Hour) - A 50 μl reaction in CutSmart <sup>TM</sup> Buffer containing 1 μg of PhiX174 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.    | Pass         |

<sup>\*</sup> The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (#R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Authorized by Derek Robinson

12 Jun 2013 nga ISO 9001





John Greci 12 Jun 2013