

New England Biolabs Certificate of Analysis

Product Name: *AflII*
Catalog #: *R0520S/L*
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 µl.*
Lot #: *0431505*
Assay Date: *05/2015*
Expiration Date: *5/2017*
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*
Specification Version: *PS-R0520S/L v1.0*
Effective Date: *26 Nov 2014*

| Assay Name/Specification (minimum release criteria) | Lot #0431505 |
|---|--------------|
| Blue-White Screening (Terminal Integrity) - A sample of Litmus28 vector linearized with a 10-fold excess of AflII, 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™ Buffer containing 1 µg of supercoiled pBR322 DNA and a minimum of 100 Units of AflII 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] <i>E. coli</i> DNA and a minimum of 100 units of AflII 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 AflII, >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 AflII. | Pass |
| 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 AflII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass |

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* 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
26 Nov 2014



Inspected by
Stephanie Doucette
27 May 2015

