

## New England Biolabs Certificate of Analysis

**Product Name:** *AflIII*  
**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 #:** *0431412*  
**Assay Date:** *12/2014*  
**Expiration Date:** *12/2016*  
**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 #0431412
<b>Blue-White Screening (Terminal Integrity)</b> - A sample of Litmus28 vector linearized with a 10-fold excess of AflIII, religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	<b>Pass</b>
<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 AflIII incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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] <i>E. coli</i> DNA and a minimum of 100 units of AflIII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 5-fold over-digestion of PhiX174 DNA with AflIII, >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 AflIII.	<b>Pass</b>
<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.	<b>Pass</b>

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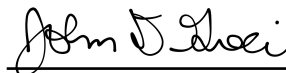
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\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for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



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Authorized by  
Derek Robinson  
26 Nov 2014



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Inspected by  
John Greci  
01 Dec 2014

