

## New England Biolabs Certificate of Analysis

*Product Name:* HpyCH4III  
*Catalog #:* R0618S/L  
*Concentration:* 5,000 units/ml  
*Unit Definition:* One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
*Lot #:* 0431804  
*Assay Date:* 04/2018  
*Expiration Date:* 4/2020  
*Storage Temp:* -20°C  
*Storage Conditions:* 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
*Specification Version:* PS-R0618S/L v2.0  
*Effective Date:* 08 Feb 2017

Assay Name/Specification (minimum release criteria)	Lot #0431804
<b>DNase Activity (Labeled Oligo, 3' extension)</b> - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 25 units of HpyCH4III incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	<b>Pass</b>
<b>DNase Activity (Labeled Oligo, 5' extension)</b> - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5' extension and a minimum of 25 units of HpyCH4III incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	<b>Pass</b>
<b>Double Stranded DNase Activity (Labeled Oligo)</b> - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 25 units of HpyCH4III incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	<b>Pass</b>
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 5-fold over-digestion of Lambda DNA with HpyCH4III, ~50% 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 HpyCH4III.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 5 units of HpyCH4III 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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<b>Assay Name/Specification</b> (minimum release criteria)	<b>Lot #0431804</b>
<b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 25 units of HpyCH4III incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	<b>Pass</b>

\* 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  
08 Feb 2017



Inspected by  
Stephanie Cornelio  
10 Apr 2018

