

## New England Biolabs Certificate of Analysis

**Product Name:** HpyCH4III  
**Catalog Number:** R0618L  
**Concentration:** 5,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10198135  
**Expiration Date:** 06/2025  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0618S/L v3.0

HpyCH4III Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0618LVIAL	HpyCH4III	10196898	Pass
B6004SVIAL	rCutSmart™ Buffer	10193042	Pass

Assay Name/Specification	Lot # 10198135
<b>DNase Activity (Labeled Oligo, 3' extension)</b> A 50 µl reaction in rCutSmart™ 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.	Pass
<b>DNase Activity (Labeled Oligo, 5' extension)</b> A 50 µl reaction in rCutSmart™ 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.	Pass
<b>Double Stranded DNase Activity (Labeled Oligo)</b> A 50 µl reaction in rCutSmart™ 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.	Pass

Assay Name/Specification	Lot # 10198135
<p><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, &gt;95% can be recut with HpyCH4III.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in rCutSmart™ 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> HpyCH4III is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> A 50 µl reaction in rCutSmart™ 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 5 units of HpyCH4III is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p>	<b>Pass</b>

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

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YunJie Sun  
Production Scientist  
27 Jun 2023



Michael Tonello  
Packaging Quality Control Inspector  
14 Jul 2023