

New England Biolabs Certificate of Analysis

Product Name: *HpyCH4III*
Catalog Number: *R0618S*
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: *10238671*
Expiration Date: *03/2026*
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*

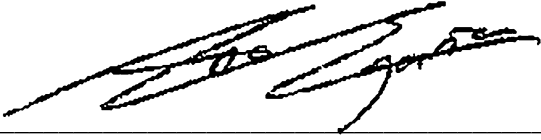
| HpyCH4III Component List | | | |
|--------------------------|-----------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| R0618SVIAL | HpyCH4III | 10233798 | Pass |
| B6004SVIAL | rCutSmart™ Buffer | 10233336 | Pass |

| Assay Name/Specification | Lot # 10238671 |
|--|----------------|
| <p>DNase Activity (Labeled Oligo, 3' extension) 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.</p> | Pass |
| <p>DNase Activity (Labeled Oligo, 5' extension) 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.</p> | Pass |
| <p>Double Stranded DNase Activity (Labeled Oligo) 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.</p> | Pass |

| Assay Name/Specification | Lot # 10238671 |
|---|----------------|
| <p>Ligation and Recutting (Terminal Integrity) 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.</p> | Pass |
| <p>Non-Specific DNase Activity (16 Hour) 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> | Pass |
| <p>Protein Purity Assay (SDS-PAGE) HpyCH4III is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p> | Pass |
| <p>Single Stranded DNase Activity (FAM-Labeled Oligo) 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 <5% degradation as determined by capillary electrophoresis.</p> | Pass |
| <p>qPCR DNA Contamination (E. coli Genomic) 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> | Pass |

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

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Production Scientist
03 Apr 2024



Michael Tonello
Packaging Quality Control Inspector
03 Apr 2024