

New England Biolabs Product Specification

<i>Product Name:</i>	<i>HpyCH4III</i>
<i>Catalog #:</i>	<i>R0618S/L</i>
<i>Concentration:</i>	<i>5,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>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)</i>
<i>Specification Version:</i>	<i>PS-R0618S/L v3.0</i>
<i>Effective Date:</i>	<i>11 Jul 2023</i>

Assay Name/Specification (minimum release criteria)

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.

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.

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.

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.

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.

Protein Purity Assay (SDS-PAGE) - HpyCH4III is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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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.

Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50 μ l 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.

*One or more products referenced in this document may be covered by a 3rd-party trademark.
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Date 11 Jul 2023

Nancy Considine
Quality Approver

