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## New England Biolabs Certificate of Analysis

Product Name: Hphl
Catalog Number: R0158L
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: 10128093
Expiration Date: 11/2023
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol,

500 μg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0158S/L/V v3.0

Hphl Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R0158LVIAL	Hphl	10128084	Pass	
B6004SVIAL	rCutSmart™ Buffer	10119384	Pass	

Assay Name/Specification	Lot # 10128093
Ligation and Recutting (Terminal Integrity) After a 5-fold over-digestion of Lambda DNA with HphI, ~50% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, ~75%	Pass
can be recut with Hphl.  Exonuclease Activity (Radioactivity Release)	Pass
A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ ³H] E. coli DNA and a minimum of 5 units of HphI incubated for 4 hours at 37°C releases <0.2% of the total radioactivity.	1 200
Protein Purity Assay (SDS-PAGE) HphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of Hphl incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass



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Assay Name/Specification	Lot # 10128093
qPCR DNA Contamination (E. coli Genomic) A minimum of 5 units of HphI 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.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 25 units of HphI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Penghua Zhang Production Scientist

01 Dec 2021

Michael Tonello

Packaging Quality Control Inspector

01 Dec 2021



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