

New England Biolabs Certificate of Analysis

Product Name: *HphI*
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: *10226027*
Expiration Date: *09/2025*
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*

HphI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0158LVIAL	HphI	10209070	Pass
B6004SVIAL	rCutSmart™ Buffer	10224839	Pass

Assay Name/Specification	Lot # 10226027
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ H] 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.	Pass
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of HphI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
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% can be recut with HphI.	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	Pass


Assay Name/Specification	Lot # 10226027
detectable nuclease degradation as determined by agarose gel electrophoresis.	
Protein Purity Assay (SDS-PAGE) HphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
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

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

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YunJie Sun
Production Scientist
04 Oct 2023



Michael Tonello
Packaging Quality Control Inspector
02 Feb 2024