

## New England Biolabs Certificate of Analysis

**Product Name:** *HphI*  
**Catalog Number:** *R0158S*  
**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:** *10157698*  
**Expiration Date:** *08/2024*  
**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
R0158SVIAL	HphI	10157697	Pass
B6004SVIAL	rCutSmart™ Buffer	10156426	Pass

Assay Name/Specification	Lot # 10157698
<b>Functional Testing (15 minute Digest)</b> 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
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> 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
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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
<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	Pass

Assay Name/Specification	Lot # 10157698
<p>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.</p>	
<p><b>Protein Purity Assay (SDS-PAGE)</b> HphI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Ligation and Recutting (Terminal Integrity)</b> 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.</p>	<b>Pass</b>

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

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