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New England Biolabs Certificate of Analysis

Product Name: Hhal
Catalog Number: R0139L
Concentration: 20,000 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: 10152716
Expiration Date: 06/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200

μg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0139S/L v2.0

Hhal Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R0139LVIAL	Hhal	10152715	Pass	
B6004SVIAL	rCutSmart™ Buffer	10149689	Pass	

Assay Name/Specification	Lot # 10152716
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 60 units of Hhal incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	
Thouse at 67 6 Toloadoc 30.17% of the total radioactivity.	
Functional Testing (15 minute Digest)	Pass
A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of Hhal	
incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	
agaiose gel electrophoresis.	
Non-Specific DNase Activity (16 Hour)	Pass
A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of	
60 units of Hhal incubated for 16 hours at 37°C results in a DNA pattern free of	
detectable nuclease degradation as determined by agarose gel electrophoresis.	
qPCR DNA Contamination (E. coli Genomic)	Pass
A minimum of 20 units of Hhal 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	



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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.	
Ligation and Recutting (Terminal Integrity) After a 10-fold over-digestion of Lambda DNA with Hhal, >95% 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 Hhal.	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**

12 Jul 2022

Michael Tonello

Packaging Quality Control Inspector

12 Jul 2022



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