

New England Biolabs Certificate of Analysis

Product Name: *MluI-HF®*
Catalog Number: *R3198S*
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: *10261450*
Expiration Date: *07/2026*
Storage Temperature: *-20°C*
Storage Conditions: *10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml rAlbumin, 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version: *PS-R3198S/L v2.0*

MluI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3198SVIAL	MluI-HF®	10249959	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10250204	Pass
B6004SVIAL	rCutSmart™ Buffer	10250208	Pass

Assay Name/Specification	Lot # 10261450
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 60 units of MluI-HF® incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
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 100 units of MluI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Functional Test (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of MluI-HF® 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 20-fold over-digestion of Lambda DNA with MluI-HF®, >95% of the DNA	Pass

Assay Name/Specification	Lot # 10261450
fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with Mlul-HF®.	
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of Mlul-HF® incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Protein Purity Assay (SDS-PAGE) Mlul-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of Mlul-HF® 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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Nancy Considine
Production Scientist
03 Sep 2024



Josh Hersey
Packaging Quality Control Inspector
05 Nov 2024