

## New England Biolabs Certificate of Analysis

**Product Name:** *MluI-HF®*  
**Catalog Number:** *R3198L*  
**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:** *10257240*  
**Expiration Date:** *08/2026*  
**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*

<b>MluI-HF® Component List</b>			
<b>NEB Part Number</b>	<b>Component Description</b>	<b>Lot Number</b>	<b>Individual QC Result</b>
R3198LVIAL	MluI-HF®	10249960	<b>Pass</b>
B7024AVIAL	Gel Loading Dye, Purple (6X)	10236426	<b>Pass</b>
B6004SVIAL	rCutSmart™ Buffer	10245415	<b>Pass</b>

<b>Assay Name/Specification</b>	<b>Lot # 10257240</b>
<b>Endonuclease Activity (Nicking)</b> 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.	<b>Pass</b>
<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 100 units of MluI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Test (15 minute Digest)</b> 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.	<b>Pass</b>
<b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of Lambda DNA with MluI-HF®, >95% of the DNA	<b>Pass</b>

Assay Name/Specification	Lot # 10257240
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®.	
<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 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> Mlul-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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.	<b>Pass</b>

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  
19 Sep 2024