240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
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www.neb.com
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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:	*10257241*
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)	10236425	**Pass**
B6004SVIAL	rCutSmart™ Buffer	10245418	**Pass**

Assay Name/Specification	**Lot # 10257241**
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 37ºC 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**

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Assay Name/Specification	Lot # 10257241
fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with MluI-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 MluI-HF® incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE) MluI-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 MluI-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.

Nancy Considine
Production Scientist
03 Sep 2024

Michael Tonello
Packaging Quality Control Inspector
06 Sep 2024