### New England Biolabs

#### Product Specification

**Product Name:** MluI  
**Catalog #:** R0198S/L  
**Concentration:** 10,000 units/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in NEBuffer™ r3.1 in 1 hour at 37°C in a total reaction volume of 50 µl.

**Shelf Life:** 24 months  
**Storage Temp:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)

**Specification Version:** PS-R0198S/L v2.0  
**Effective Date:** 03 Mar 2022  

#### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of MluI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of MluI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (15 minute Digest)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of MluI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

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

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 100 units of MluI 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)** - A minimum of 10 units of MluI 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.
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