

## New England Biolabs Certificate of Analysis

Product Name: LunaScript<sup>®</sup> RT SuperMix  
 Catalog Number: M3010L  
 Concentration: 5 X Concentrate  
 Packaging Lot Number: 10184152  
 Expiration Date: 06/2024  
 Storage Temperature: -20°C  
 Specification Version: PS-M3010S/L/X/E v2.0  
 Composition (1X): Proprietary

LunaScript <sup>®</sup> RT SuperMix Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M3010LVIAL	LunaScript <sup>®</sup> RT SuperMix	10151266	Pass

Assay Name/Specification	Lot # 10184152
<p><b>Endonuclease Activity (Nicking)</b>            A 50 µl reaction in ThermoPol<sup>®</sup> Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of Luna<sup>®</sup> Reverse Transcriptase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Functional Testing (Two-Step RT-qPCR)</b>            The LunaScript<sup>®</sup> RT SuperMix is functionally tested in two-step RT-qPCR with human RNA template, resulting in a standard curve with a calculated qPCR efficiency of 90-110%, and a dynamic range of 7 orders of magnitude.</p>	Pass
<p><b>Phosphatase Activity (pNPP)</b>            A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units of Luna<sup>®</sup> Reverse Transcriptase incubated for 4 hours at 37°C yields &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	Pass
<p><b>Protein Purity Assay (SDS-PAGE)</b>            Luna<sup>®</sup> Reverse Transcriptase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>RNase Activity Assay (4 Hour Digestion)</b>            A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA</p>	Pass

Assay Name/Specification	Lot # 10184152
<p>and a minimum of 100 units of Luna<sup>®</sup> Reverse Transcriptase is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	
<p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> A 50 µl reaction in CutSmart<sup>®</sup> Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 250 units of Luna<sup>®</sup> Reverse Transcriptase incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 1 µl of LunaScript<sup>®</sup> RT SuperMix is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> 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.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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