NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*LunaScript® RT SuperMix*
Catalog #:	*M3010S/L/X/E*
Concentration:	*5X Concentrate*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Composition (1X):	*Proprietary*
Specification Version:	*PS-M3010S/L/X/E v2.0*
Effective Date:	*13 Jun 2022*

Assay Name/Specification (minimum release criteria)
Endonuclease Activity (Nicking) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of Luna® Reverse Transcriptase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units of Luna® Reverse Transcriptase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.
Protein Purity Assay (SDS-PAGE) - Luna® Reverse Transcriptase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 1 µl of LunaScript® RT SuperMix 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.
RNase Activity Assay (4 Hour Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 100 units of Luna® Reverse Transcriptase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.
Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 250 units of Luna® Reverse Transcriptase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

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Assay Name/Specification (minimum release criteria)
Functional Testing (Two-Step RT-qPCR) - The LunaScript® 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.

Date 13 Jun 2022

Derek Robinson
Director, Quality Control