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LunaScript® RT SuperMix Kit

NEB #E3010S/L

25/100 reactions

Version 2.0_1/20

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Kit Components

This product should be stored at –20°C, protected from light, and has a shelf-life of 2 years. The LunaScript RT SuperMix and No-RT Control Mix usually remain unfrozen at -20°C. The components are stable for at least 30 freeze/thaw cycles, and for short-term storage may be stored at 4°C for up to 1 month.

All components are provided in volumes sufficient for preparation of up to 25 reactions (NEB #E3010S), 100 reactions (NEB #E3010L). Nuclease-free Water is supplied in 1.5 ml aliquots.

LunaScript RT SuperMix (5X)

No-RT Control Mix (5X)

Nuclease-free Water

Required Materials Not Included

RNA template

PCR strip tubes or microcentrifuge tubes

Thermocycler

Introduction

LunaScript RT SuperMix is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna® Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is also included to protect template RNA from degradation. The LunaScript SuperMix contains random hexamer and poly-dT primers, allowing even coverage across the length of the RNA targets. In addition, the LunaScript RT SuperMix contains a blue dye, providing a visual indicator that can be followed throughout the two-step RT-qPCR process. The LunaScript RT SuperMix offers robust, linear, and sensitive detection using total RNA inputs as high as 1 µg and as low as single copies of RNA.

General Tips and Considerations

Template RNA

- RNA of high purity enables the most sensitive RT-qPCR assays (1,2).
- Total RNA or mRNA can be used as input for first strand cDNA synthesis. Total RNA is generally sufficient for most RT-qPCR experiments and can be prepared using typical column-based methods, e.g. Monarch® RNA Miniprep Kit (NEB #T2010). If desired, mRNA can be easily purified using a polyA Spin™ mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).

- The amount of RNA required for detection depends on the abundance of the transcript-of-interest. In general, 1 ng to 1 µg total RNA or 0.1 ng to 100 ng mRNA are recommended.

cDNA Synthesis

- The LunaScript RT SuperMix can perform cDNA synthesis in the temperature range of 45°C to 65°C. 55°C is optimal for most applications.
- In general, we recommend the transfer of 1 µl cDNA product into a 20 µl qPCR experiment. Where useful, up to 20% qPCR volume can be undiluted cDNA product (e.g. 4 µl cDNA product into a 20 µl Luna qPCR reaction without significantly compromising performance).
- The presence of genomic DNA or carry-over products can interfere with the accurate quantitation of target RNA, particularly for low copy targets. Therefore, it is important to carry out the appropriate "No-RT" control reactions to account for these effects. In addition, no template control (NTC) reactions should be set up to demonstrate that positive reactions are meaningful.

LunaScript RT SuperMix Kit Protocols

1. Mix components briefly and spin down if necessary.
2. Prepare cDNA synthesis reaction as described below:

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
LunaScript RT SuperMix (5X)	4 µl	1X
RNA Sample	variable	(up to 1 µg)*
Nuclease-free Water	to 20 µl	

For no-RT control reactions, mix the following components:

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
No-RT Control Mix (5X)	4 µl	1X
RNA Sample	variable	(up to 1 µg)*
Nuclease-free Water	to 20 µl	

*Up to 1 µg total RNA, 1 µg mRNA or 100 ng specific RNA can be used in a 20 µl reaction.
However, the cDNA input for downstream qPCR detection should typically contain $< 10^{9}$ copies of the target to ensure that quantitation remains linear. To accommodate larger amounts of input RNA (> 1 µg), the reaction should be scaled up to ensure optimum cDNA synthesis.

For no template controls, mix the following components:

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
LunaScript RT SuperMix (5X)	4 µl	1X
Nuclease-free Water	16 µl	

Incubate reactions in a thermocycler with the following steps:

CYCLE STEP	TEMP	TIME	CYCLES
Primer Annealing	25°C	2 minutes	
cDNA Synthesis	55°C	10 minutes	1
Heat Inactivation	95°C	1 minute	

Troubleshooting Guide

Note: For additional assistance please refer to product FAQ's at www.neb.com/E3010.

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Low cDNA yield	Insufficient quality or quantity of starting material	• Check the integrity of the RNA using denaturing agarose gel electrophoresis (2) or BioAnalyzer® (Agilent Technologies) • RNA should have a minimum A_{260}/A_{280} ratio of 1.8 or higher. Ethanol precipitation followed by a 70% ethanol wash can remove most contaminants such as EDTA and guanidinium. Precipitation with lithium chloride can remove polysaccharides (2). • Phenol/chloroform extraction and ethanol extraction can remove contaminant proteins such as proteases (2) • Use sufficient amount of input RNA

References

1. Fleige, S. and Pfaffl (2006) *Molecular Aspects of Medicine* 27, 126–139.
2. Sambrook, J. and Russel, D.W. (2001) Molecular Cloning: A Laboratory Manual (3rd Ed.) *Cold Spring Harbor: Cold Spring Harbor Laboratory Press*.

Ordering Information

NEB #	**PRODUCT**	**SIZE**
E3010S/L	LunaScript RT SuperMix Kit	25/100 reactions

COMPANION PRODUCTS

NEB #	**PRODUCT**	**SIZE**
M3003S/L	Luna Universal qPCR Master Mix	100/500 reactions
M3003X	Luna Universal qPCR Master Mix	1,000 reactions
M3003E	Luna Universal qPCR Master Mix	2,500 reactions
M3004S/L	Luna Universal Probe qPCR Master Mix	200/500 reactions
M3004X	Luna Universal Probe qPCR Master Mix	1,000 reactions
M3004E	Luna Universal Probe qPCR Master Mix	2,500 reactions
T2010S	Monarch RNA Miniprep Kit	50 preps
S1560S	polyA Spin mRNA Isolation Kit	8 isolations
S1550S	Magnetic mRNA Isolation Kit	25 isolations

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	1/18
2.0	Update to new manual format	1/20

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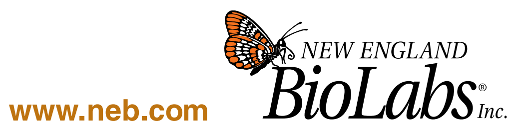

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