INSTRUCTION MANUAL

LunaScript® RT Master Mix Kit (Primer-free)

NEB #E3025S/L

25/100 reactions

Version 1.0_4/21

Table of Contents

Kit Components

This product should be stored at –20°C and has a shelf-life of 2 years. The LunaScript RT Master Mix (Primer-free) and No-RT Control Mix (Primer-free) 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 week.

All components are provided in volumes sufficient for preparation of up to 25 reactions (NEB #E3025S), 100 reactions (NEB #E3025L). Nuclease-free Water is supplied in 1.5 ml aliquots.

LunaScript RT Master Mix (Primer-free) (5X)

No-RT Control Mix (Primer-free) (5X)

Nuclease-free Water

Required Materials Not Included

RNA template

RT primers

PCR strip tubes or microcentrifuge tubes

Thermocycler

Introduction

LunaScript RT Master Mix Kit (Primer-free) is an optimized master mix containing all the necessary components for first strand cDNA synthesis except for primers. The mix is compatible with random primers, oligo dT primers, and gene-specific primers enabling maximum cDNA synthesis flexibility. LunaScript RT Master Mix (Primer-free) features the thermostable Luna® Reverse Transcriptase which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is included to protect template RNA from degradation. In addition, the presence of a blue dye provides a visual indicator for the RT step as well as for downstream applications.

The cDNA product generated by the LunaScript RT Master Mix (Primer-free) can be used in a variety of downstream applications. For real-time PCR quantitation, the LunaScript RT Master Mix (Primer-free) is recommended to generate cDNA in the presence of a mixture of random and dT primers. It offers robust, linear, and sensitive detection from up to 1 µg total RNA input and down to low copies of RNA, similar to LunaScript RT SuperMix Kit (NEB #E3010). To generate long or full-length cDNAs, oligo dT primers should be used. With a short incubation at 55ºC for 10 minutes, the LunaScript RT Master Mix (Primer-free) can make cDNA products up to 9 kb.

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 reactions. Total RNA is generally sufficient for first strand cDNA synthesis reactions and can be prepared using typical column-based methods, e.g., Monarch® Total RNA Miniprep Kit (NEB #T2010). If desired, mRNA can be purified using the Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for detection depends on the abundance of the transcript-of-interest. As a starting point, 1 ng to 1 µg total RNA or 0.1 ng to 100 ng mRNA is recommended.

cDNA Synthesis

- The LunaScript RT Master Mix (Primer-free) can perform cDNA synthesis in the temperature range of 45°C to 65°C. An RT step of 55°C is optimal for most applications.
- A variety of primers can be added to the LunaScript RT Master Mix (Primer-free) to enable first strand cDNA synthesis, including random primers, oligo dT primers, or gene-specific primers. The Random Primer Mix from NEB (a mixture of random hexamers and anchored dT primers, NEB #S1330) is recommended for cDNA synthesis prior to real-time qPCR detection. Oligo $d(T)_{23}VN$ (NEB #S1327) is recommended if long or full-length cDNA synthesis is desired. When gene-specific primers are used, the primer sequence may need to be optimized for optimal priming efficiency.

APPLICATION	FULL-LENGTH OR LONG cDNA SYNTHESIS	cDNA SYNTHESIS UPSTREAM OF qPCR	TARGET SPECIFIC cDNA SYNTHESIS
Primer	Oligo $d(T)_{23}VN$	Random Primer Mix	Specific Primer
Final Concentration	5 µM	6 µM	0.5 µM (Range: 0.1-1 µM)

- In general, we recommend the transfer of 1 µl cDNA product into a 20 µl qPCR or PCR experiment. Where needed, up to 20% qPCR/PCR volume can be undiluted cDNA product (e.g., 4 µl cDNA product into a 20 µl qPCR reaction without significantly compromising performance).
- The presence of genomic DNA or carryover products from previous amplification 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 Master Mix Kit Protocols

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

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
LunaScript RT Master Mix (Primer-free) (5X)	4 µl	1X
Random Primer Mix (60 µM)[1] Or d(T)$_{23}$VN (50 µM) [2] Or Gene-specific primer[3]	2 µl 2 µl x µl	6 µM 5 µM 0.1–1 µM (typically 0.5 µM final)
RNA Sample[4]	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 (Primer-free) (5X)	4 µl	1X
Random Primer Mix (60 µM)[1] Or d(T)$_{23}$VN (50 µM) [2] Or Gene-specific primer[3]	2 µl 2 µl x µl	6 µM 5 µM 0.1–1 µM (typically 0.5 µM final)
RNA Sample[4]	variable	(up to 1 µg)
Nuclease-free Water	to 20 µl	

For no template controls, mix the following components:

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
LunaScript RT Master Mix (Primer-free) (5X)	4 µl	1X
Random Primer Mix (60 µM)[1] Or d(T)$_{23}$VN (50 µM) [2] Or Gene-specific primer[3]	2 µl 2 µl x µl	6 µM 5 µM 0.1–1 µM (typically 0.5 µM final)
Nuclease-free Water	to 20 µl	

[1] Random Primer Mix (NEB #S1330) is recommended for real-time qPCR detection.

[2] Oligo d(T)$_{23}$VN (NEB #S1327) is recommended for long or full-length cDNA synthesis.

[3] Gene-specific primers can be used for target-specific cDNA synthesis. The final concentration is typically 0.5 µM and can be optimized in the range of 0.1–1 µM.

[4] Up to 1 µg total RNA, 1 µg mRNA or 100 ng specific RNA can be used in a 20 µl reaction. To accommodate larger amounts of input RNA (> 1 µg), the reaction should be scaled up to ensure optimum cDNA synthesis.

Incubate reactions in a thermocycler with the following steps:

PRIMERS FOR cDNA SYNTHESIS	CYCLE STEP	TEMP	TIME	CYCLES
Random Primers (e.g., Random Primer Mix)	Primer Annealing	25°C	2 minutes	1
	cDNA Synthesis	55°C	10 minutes	
	Heat Inactivation	95°C	1 minute	
Oligo-dT primers or a gene-specific primer	cDNA Synthesis	55°C	10 minutes	1
	Heat Inactivation	95°C	1 minute	

The cDNA product should be stored at -20°C. In general, the volume of cDNA product should not exceed 1/10 of the qPCR or PCR reaction volume. Where needed, up to 20% qPCR volume can be undiluted cDNA product.

For qPCR applications, we recommend using Luna Universal qPCR Master Mix (NEB #M3003) for dye-based qPCR detection and Luna Universal Probe qPCR Master Mix (NEB #M3004) for probe-based detection.
For downstream PCR, we recommend One*Taq*® 2X Master Mix (NEB #M0482 or NEB #M0485) for PCR detection up to 5 kb, Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494) for highest fidelity, and LongAmp® *Taq* 2X Master Mix (NEB #M0287) for high yields from longer products.

Troubleshooting Guide

Note: For additional assistance please refer to product FAQs at www.neb.com/E3025.

PROBLEM		POSSIBLE CAUSE(S)	SOLUTION(S)
First Strand cDNA Synthesis	Low cDNA yield	Low quality of RNA templates Insufficient quantity of starting material	• Check the integrity of the RNA using denaturing agarose gel electrophoresis (2) or BioAnalyzer® • 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) • Repurify RNA samples using column-based methods e.g., Monarch Total RNA Miniprep Kit • Use sufficient amount of input RNA
2 Step RT-qPCR	Poor linearity	Poor RNA integrity Inhibitors in the RNA templates Too much cDNA added in the qPCR experiments	• Ensure the purity and quality of the RNA templates • Repurify RNA samples • Add < 20% cDNA to the amplification reaction
	Positive signals in the NRT reactions	DNA contamination in the RNA templates	• Perform DNase I treatment to remove DNA contaminants • Use qPCR primers spanning exons
	Positive signals in the NTC reactions	Contamination in the reagents used in the cDNA synthesis and/or qPCR steps	• Follow good laboratory RT-qPCR practices • Replace the contaminated reagent(s)
2 Step RT-qPCR	Low yield of RT-PCR product	Poor RNA integrity Inhibitors in the RNA templates Insufficient quantity of starting material Suboptimal PCR reaction conditions	• Ensure the purity and quality of the RNA templates • Repurify RNA samples • Use sufficient amount of input RNA • Optimize PCR reactions, e.g., primers, annealing temperature; use < 10% cDNA in the PCR reactions

References

1. Fleige, S. and Pfaffl, M. (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
E3025S/L	LunaScript RT Master Mix Kit (Primer-free)	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
E3010S/L	LunaScript RT SuperMix Kit	25/100 reactions
E6560S/L	ProtoScript® II First Strand cDNA Synthesis Kit	30/150 reactions
T2010S	Monarch Total RNA Miniprep Kit	50 preps
S1550S	Magnetic mRNA Isolation Kit	25 isolations
S1330S	Random Primer Mix	100 µl
S1327S	Oligo $d(T)_{23}VN$	1 A_{260} unit

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	4/21

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