NEW ENGLAND Biolabs

LunaScript RT Master Mix Kit Protocols (NEB #E3025)

Overview

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

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
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 5kb, 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.