Protocol for LunaScript® RT SuperMix Kit (E3010)

1. Mix components briefly and spin down if necessary.

2. Prepare cDNA synthesis reaction as described below:

COMPONENTS	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 reaction, mix the following components:

COMPONENTS	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:

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

Incubate reactions in a thermocycler with the following steps:

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

Links to this resource

Related Products: LunaScript™ RT SuperMix Kit