Protocol for Two-step RT-qPCR using the LunaScript® RT SuperMix Kit (NEB #E3010) and the Luna® Universal qPCR Master Mix (NEB #M3003) or Luna Universal Probe qPCR Master Mix (NEB #M3004)

Step 1: First strand cDNA synthesis

1. Mix components briefly and spin down if necessary.
Note: LunaScript RT SuperMix and No-RT Control Mix are usually not frozen at -20°C.

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 (up to 1 µg)*	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.

3. 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	1
Heat Inactivation	95°C	1 minute	1

Step 2: qPCR reaction

The cDNA product can be directly used in qPCR reaction. In general, 1 µl cDNA product is recommended for usage in a 20 µl qPCR detection. When necessary, up to 20% qPCR volume can be undiluted cDNA product (e.g., 4 µl cDNA product in a 20 µl qPCR reaction).

We recommend using the Luna® Universal qPCR Master Mix (NEB #M3003) and Luna Universal Probe qPCR Master Mix (NEB #M3004) for qPCR detection, as cDNA products generated using the LunaScript RT SuperMix Kit have been extensively evaluated using the two Luna kits.

- Prepare dye-based qPCR detection as follows:

Component	20 µl Reaction	Final Concentration
Luna Universal qPCR Master Mix (NEB #M3003)	10 µl	1X
10 µM forward primer	0.5 µl	0.25 µM
10 µM reverse primer	0.5 µl	0.25 µM
cDNA products	1 µl	< 4 µl
Nuclease-free water	to 20 µl	

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	60 seconds	1
Denaturation Extension	95 °C 60 °C	15 seconds 30 seconds	40-45
Melt Curve	60–95°C		1

- Reaction setup for probe-based qPCR detection

Component	20 µl Reaction	Final Concentration
Luna Universal Probe qPCR Master Mix (NEB #M3004)	10 µl	1X
10 µM forward primer	0.8 µl	0.4 µM
10 µM reverse primer	0.8 µl	0.4 µM
10 µM probe	0.4 µl	0.2 µM
cDNA products	1 µl	< 4 µl
Nuclease-free water	to 20 µl	

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	60 seconds	1
Denaturation Extension	95 °C 60 °C	15 seconds 30 seconds	40-45

Links to this resource

Related Products: LunaScript™ RT SuperMix Kit, Luna® Universal qPCR Master Mix, Luna® Universal Probe qPCR Master Mix