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## RT-qPCR Protocol Part

**Important notes before getting started**

- **Thaw One-Step RT-qPCR 2X master mix and primer solutions** on ice.
  Keep the solutions on ice after complete thawing.
  Mix well before use to avoid localized differences in salt concentration.

**Recommended protocols for RT-qPCR:**

Add the following components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the remaining components are added.

| Component | Stock conc. | Final conc., | 20µL assay | 50µL assay |
|---|---|---|---|---|
| RNA template | - | up to100ng polyA RNA or total RNA | x µL | x µL |
| forward Primer | 10µM | 400nM * | 0.8µL | 2µL |
| reverse Primer | 10µM | 400nM * | 0.8µL | 2µL |
| PCR-grade water | - | - | fill up to 20µL | fill up to 50µL |
| One-Step RT-qPCR 2X master mix ** | 2X | 1X | 10µL | 25µL |

* The optimal concentration for primers may vary from 100nM to 500nM

** The One-Step RT-qPCR 2X master mix contains already RNase inhibitor that may be essential when working with low amounts of starting RNA.

Continue with the RT-qPCR protocol as recommended (see below).

**Reverse transcription and thermal cycling**

Place the vials of the RT-qPCR mix in a PCR cycler and start the following program.

| Step | Temperature | Incubation | Repeats |
|---|---|---|---|
| 1. Reverse transcription | 50°C to 55** | 10-15 min. * | 1x |
| 2. Initial denaturation | 95°C | 5 min. *** | 1x |
| 3a. denaturation<br>3b. annealing<br>3c. elongation | 95°C<br>60-65°C ****<br>72°C | 15 sec<br>20 sec.<br>1 min/kb ***** | 35-45x |

* A reverse transcription time of 10 min is recommended for optimal amplicon lengths between 100bp and 200bp. Longer amplicons up to 500 bp may require a prolonged incubation of 15 min.
** The optimal temperature depends on the structural features of the RNA. Increase the temperature to 55°C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular RNA.
*** An initial denaturation time of 5 min is recommended to inactivate the reverse transcriptase!
**** The annealing temperature depends on the melting temperature of the primers and DNA probe used.
***** The elongation time depends on the length of the amplicon.
A time of 1 min for a fragment of 1,000bp is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary.

**Note**: the optimal reaction times and temperatures should be adjusted for each particular RNA / primer pair.