

**Product Information:****One step RT-PCR Master Mix (2x)** (Cat.: R2120)**Storage:** -20°C**Size:** 1.25 mL (100 Reactions)**Description:**

The ready-to-use one step RT-PCR Master Mix contains 2x M-MLV Reverse Transcriptase, Taq DNA Polymerase, recombinant RNase Inhibitor, Ultrapure nucleotides, magnesium and PCR reaction buffers, designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA. Simply mix the RNA template, primers, and RNase-free water with the RT-PCR master mix and the reactions are ready to cycles.

**Procedure:****Prepare a single reaction (total volume: 25uL) in a 0.2 or 0.5 mL microtube.**

| Component                       | Volume (μL) | Final Concentration |
|---------------------------------|-------------|---------------------|
| One step RT-PCR Master Mix (2x) | 12.5        | 1x                  |
| RNA Template                    | 0.1-1       | determined by user  |
| Forward primer (5μM)            | 1           | 200nM               |
| Reverse primer (5μM)            | 1           | 200nM               |
| PCR grade/RNase-free water      | up to 25 μL |                     |

**Setup typical thermal cycling parameters**

|                                     |      |   |
|-------------------------------------|------|---|
| 1. Reverse Transcription (RT) step: | 45°C | 15 minutes  |
| 2. Enzyme activation step:          | 95°C | 2 minutes   |
| 3. 25-40 cycles:                    |      |   |
| Denature                            | 95°C | 30 seconds  |
| Annealing                           | X°C  | 30 seconds dependent on T <sub>m</sub> of primers |
| Extension                           | 68°C | 1 minute (1min per kb amplicon)                   |
| 4. Final extension                  | 68°C | 1 minute (1min per kb amplicon)                   |
| 5. Hold 4°C                         |      |   |

**Precautions and Disclaimer:**

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.

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