

cDNA Preparation with Reverse Transcription Master Mix

Reverse Transcription Master Mix is a 5X master mix containing all the components required for cDNA synthesis, including buffer, dNTPs, primers, a ribonuclease inhibitor, and an engineered RNaseH⁺ MMLV reverse transcriptase. A mixture of random primers and oligo dT is used for priming. The cDNA prepared using the Reverse Transcription Master Mix is suitable for preamplification using Preamp Master Mix.

The range of total RNA that can be used in a 5 μ L reverse transcription reaction is 2.5 pg to 250 ng. However, the success with a given sample in qPCR depends on the level of gene expression for the genes of interest, the percentage of mRNA in the total RNA, and the number of cycles of preamplification performed prior to qPCR. In general, for total RNA input in the range of 2 ng to 250 ng, 10–14 cycles of preamplification should be sufficient. For a total RNA input less than 2 ng, increasing the number of preamplification cycles to 18–20 may improve performance.

Prepare RT Reactions

- 1 Thaw all reagents on ice. Briefly vortex and centrifuge the reagents before using.
- 2 On ice, prepare a pre-mix of the Reverse Transcription Master Mix and water as indicated in the following table:

Component	Volume per Reaction (μ L)	Volume for 48 Reactions* (μ L)	Volume for 96 Reactions* (μ L)	Volume for 192 Reactions* (μ L)
PRE-MIX				
Reverse Transcription Master Mix (Fluidigm PN 100-6297)	1.0	52.8	105.6	211.2
RNase-free water	3.0	158.4	316.8	633.6
RNA (2.5 pg/ μ L–250 ng/ μ L)	1.0			
Total	5.0	211.2	422.4	844.8

* Includes 10% overage for ease of pipetting.

- 3 In a PCR plate (on ice), aliquot 4 μ L of pre-mix for each sample.
- 4 Add 1 μ L of RNA to each well containing pre-mix, making a total volume of 5 μ L.
- 5 Properly seal and gently vortex to mix the reverse transcription reactions.
- 6 Centrifuge the reactions and place in a standard thermal cycler.

Thermal-Cycle

In a thermal cycler, incubate using the following protocol:

Condition	Temperature	Time
Hold	25 °C	5 min
Hold	42 °C	30 min
Hold	85 °C	5 min
Hold	4 °C	∞

After the reverse transcription reaction is complete, the reactions can be stored at –20 °C or used immediately for preamplification reactions with Preamp Master Mix.

The cDNA prepared with Reverse Transcription Master Mix can be used in preamplification with either TaqMan® Gene Expression Assays or Delta Gene™ assays. You can use up to 1.25 µL of the reaction in a 5 µL preamplification reaction.

Ordering Information

Part Number	Description	Volume per tube (µL)
100-6298	Reverse Transcription Master Mix—1 Tube	106
100-6299	Reverse Transcription Master Mix—5 Tubes	106
100-6300	Preamp and Reverse Transcription Master Mix—1 Tube	106
100-6301	Preamp and Reverse Transcription Master Mix—5 Tubes	106
100-5580	Preamp Master Mix—1 Tube	106
100-5581	Preamp Master Mix—5 Tubes	106

For technical support visit fluidigm.com/support.

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