

# RT Master Mix for qPCR

## 1 Contents

Contents	HY-K0510-100 rxns	HY-K0510-500 rxns
2× Super RT Mix	1 mL	1 mL × 5
RNase-Free H <sub>2</sub> O	1 mL × 2	1 mL × 10

## 2 General Information

MCE RT Master Mix for qPCR is a convenient, ready-to-use kit for reverse transcription. The 2× Super RT Mix contains all the reagents necessary for first-strand cDNA synthesis, including high-quality Reverse Transcriptase, RNase Inhibitor, Oligo dT Primer, Random Primer, RT Buffer and dNTPs Mix. With RNA added, the optimized system will provide sensitive and reliable cDNA synthesis. This formulation reduces handling errors significantly with only one transfer step and can be used with very low and very high amounts of input RNA (total RNA: 5 ng-5 µg in a 20 µL reaction). Upon completion of the first-strand cDNA synthesis, the cDNA product can be directly applied as a template in a standard PCR and real time quantitative PCR (qPCR). MCE SYBR Green qPCR Master Mix (HY-K0501) is highly recommended for detection of the expression levels of interested genes.

## 3 Protocol

1. Thaw RNA templates and the 2× Super RT Mix on ice. Mix solutions gently but thoroughly.
2. Prepare the following reaction mixture in a PCR tube on ice.

Components	Quantity
2× Super RT Mix	10 µL
Total RNA/ mRNA	5 ng-5 µg/ 5 ng-500 ng
RNase-Free H <sub>2</sub> O	To 20 µL

3. Mix the components well and collect by brief centrifugation. Incubate the mixture in a PCR instrument or water bath as follows:

Temperature	Time
25°C	5 mins
42°C	30-60 mins
85°C	2 mins

Note:

- a. For GC rich or structurally complex RNA templates, increasing the RT incubation temperature up to 50°C may improve the yields of cDNA.
  - b. Stop the reaction by heating at 85°C for 2 minutes followed by chilling on ice.
4. The newly synthesized first-strand cDNA is ready for immediate downstream applications or for long-term storage at -20°C.

## 4 Storage condition

-20°C

Avoid repetitive freeze-thaw cycles.

## 5 Precautions

1. High-quality, intact RNA is essential for accurate quantification in qPCR. RNA should be devoid of RNase contamination and RNA quality can be analyzed by agarose gel electrophoresis.
2. Prepare the reaction mixture on ice and avoid RNase contamination.
3. Always use nuclease-free, commercially autoclaved reaction tubes, sterile aerosol-resistant tips and gloves. Ensure that reagents, tubes and tips are kept RNase-free by using sterile technique.
4. 2× Super RT Mix contains glycerol. Therefore, before pipetting, please collect the liquid by a brief centrifugation.
5. To eliminate any amplification from gDNA, design primers to span the exon-exon junction. You can also choose MCE RT Master Mix for qPCR (gDNA digester plus) (HY-K0511).
6. This product is for R&D use only, not for drug, household, or other uses.
7. For your safety and health, please wear a lab coat and gloves while handling.

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