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RT Master Mix for qPCR (gDNA digester plus)

1 Contents

Contents	HY-K0511-100 rxns	HY-K0511-500 rxns
gDNA digester	100 µL	100 µL × 5
5× gDNA digester Buffer	200 µL	200 µL × 5
2× Super RT Mix	1 mL	1 mL × 5
RNase-Free H_2O	1 mL × 2	1 mL × 10

2 General Information

MCE RT Master Mix for qPCR (gDNA digester plus) is a convenient, ready-to-use kit for reverse transcription. This kit contains gDNA digester which can eliminate genomic DNA (gDNA) contaminations in RNA samples. 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. The optimized system will provide sensitive and reliable cDNA synthesis and reduce handling errors significantly. 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 higly recommended for detection of the expression levels of interested genes.

3 Protocol

1. Thaw RNA templates, gDNA digester, 5× gDNA digester Buffer 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. Mix thoroughly, and incubate at 42°C for 2 minutes.

Components	Quantity
5× gDNA digester Buffer	2 µL
gDNA digester	1 µL
Total RNA/ mRNA	5 ng-5 µg/ 5 ng-500 ng
RNase-Free H_2O	To 10 µL

3. Add 10 µL of 2× Super RT Mix (gDNA digester inhibitor contained) to the mixture from Step 2 (10 µL). Mix the components well and collect by brief centrifugation. Incubate the mixture in a PCR instrument or water bath in the procedure 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. gDNA digester, 5× gDNA digester Buffer and the 2× Super RT Mix contain 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.

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.