Version Number：1.0

RT Easy ™II

Master Premix for first-strand cDNA synthesis for Real Time PCR

Component (10μl system)	RT-01021 50 T	RT-01022 200 T	RT-01023 800T
2× RT OR-Easy™ Mix	0.25ml	1ml	1ml × 4
RNase-Free ddH_2O	1.7ml	1.7ml	1.7ml × 3
Manual	1 serving	1 serving	1 serving

Product Introduction

2× RT OR-Easy™ Mix can synthesize the first-strand cDNA at a temperature as high as 50 ℃ , which is conducive to the reverse transcription reaction of complex secondary structure RNA templates.

The RNA purified in the laboratory often contains alcohol and guanidine salt residues, which have strong inhibitory properties on most reverse transcriptase, resulting in unsatisfactory reverse transcription effects or low reverse transcription efficiency. 2×RT Mix shows extremely high tolerance to alcohol and guanidine salt. The highest tolerance to alcohol in RNA samples is 60%, and the highest tolerance to guanidine salt is 750 mM. Even impure RNA can be used for reverse transcription reaction with 2× RT OR-Easy™ Mix .

2× RT OR-Easy™ Mix is a rapid reverse transcription reagent specially developed for Real Time PCR . This system has high reverse transcription efficiency and does not need to add any primers, and can perform good reverse transcription reactions on a small amount of RNA templates. The unique reaction system makes the RT reaction easier, faster and more efficient. The first strand cDNA synthesis can be completed in 20 minutes. This product can be used in conjunction with Foregene 's Real Time PCR Easy™ MIX to obtain high-quality experimental results.

Transport and storage conditions

1. Transportation conditions: The whole process is transported in a low-temperature ice box to ensure that the kit is in a state of <4 °C.
2. Storage conditions: stored at -20 ℃ .

Kit component information

2× RT OR-Easy ™ Mix : Foregene Reverse Transcriptase, RNase Inhibitor, dNTPs, stabilizers, enhancers, optimizers, and optimized reverse transcription primers (Random Primer, Oligo (dT)$_{18}$ Primer).

Precautions:

◆ It is recommended to use RNA extracted from fresh samples or stored at -80℃ as the template (RNA should avoid repeated freezing and thawing).

◆ Order to avoid RNase contamination, the experiment operation should be carried out in the RNase-Free space; the pipette tips and PCR centrifuge tubes used must be RNase-Free; Disposable gloves and masks should be worn.

◆ Preparation before experiment, put the 2× RT OR- Easy ™ Mix on ice to completely melt, flick and mix well before use; please operate the system on an ice bath to improve the performance of the kit.

◆ 2× RT OR- Easy ™ Mix has already added reverse transcription primers with optimized ratios, so there is no need to add any additional primers.

Template RNA dosage

Template RNA is preferably used fresh sample extracted or -80℃ stored under RNA (RNA should avoid repeated freezing and thawing) .

RT the Easy ™ II: (0.1 pg- of 0. 5 ug Total RNA or 0.01 pg - 0.0 5 ug the mRNA) / 10 μl system.

Protocol for First Strand cDNA Synthesis

A: Preparation of materials and reagents

1. Prepare RNA template (it is recommended to use Foregene Total RNA Isolation Kit) and related consumables and instruments.

   Note: As the template RNA， please make sure that the RNA is not degraded.

2. Take out 2× RT OR- Easy ™ Mix and place it on an ice bath, let it melt naturally, and gently knead it for later use; take out RNase-Free ddH_2O and melt it and place it on an ice bath for later use.

B: RT system preparation

2× RT OR- Easy ™ Mix is convenient and quick to use, avoiding pollution during operation and experimental errors caused by multiple preparations of the reaction system to the greatest extent. Use only half the volume of the reaction system (for example, if the reaction system is 10 μl, take 5 μl 2× RT OR- Easy ™ Mix), add RNA template, and add RNase-Free ddH_2O to make up the volume to 10 μl. The specific RT reaction system preparation can refer to Table 1 below.

Table 1: RT system preparation

RT system add content	With the amount
2× RT OR- Easy ™ Mix	5 μl
Template (RNA)	X μl (Total RNA:< 0.5μg / mRNA:<0.05 μg)
RNase-Free ddH_2O	(5 -X) μl
Total Volume	10 μl

C: RT reaction program setting

1. After preparing the RT system with reference to the above table, mix gently (you can use a pipette tip to blow gently; you can also mix it on a vortexer and centrifuge it to collect the liquid scattered on the tube wall or tube lid, and place it in an ice box on standby).

2. Refer to the RT reaction program below to set the reaction temperature, time, etc. (Table 2).

   Note: In order to ensure the activity of 2× RT OR- Easy TM Mix and increase its amplification efficiency, it is best to wait until the metal bath reaches the set reaction temperature (reverse transcription temperature 42 ℃ , 50 ℃ or 65 ℃ , according to the secondary structure of RNA template Choose the appropriate reaction temperature) and then prepare the RT reaction system, so that the reaction program can be entered immediately after the system preparation is completed. The RT reaction can also be performed on a PCR machine.

3. After the reaction is complete, put it on ice and use it directly for Real Time PCR, or store at -20 ℃ for up to a week. Long-term storage should be placed at -80 ℃ to avoid repeated freezing and thawing.

4. Table 2: RT reaction program settings

Step	Temperature	Time	Content
1	42 ℃	15 min	Reverse transcription
2	85 ℃	5min	Inactivation

Note: The above procedure is for reference only. The actual reaction conditions vary depending on the structural conditions of the template, primers, etc. Typically the initial reaction temperatures suggested 42 ℃ ; having two complex structures of the RNA template , a first step the reaction temperature is recommended to use 50℃or 65℃.