FOREGENE

RT Easy™ II(With gDNase)

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

Cat.No.RT-01031/01032

Fast and highly sensitive reverse transcription system for generating first-strand cDNA for use in Real Time PCR

For research use only

Store at -20℃

Product Description

RT Easy™ II (With gDNase) is a reverse transcription system specially developed for Real Time PCR to rapidly remove contamination from genomic DNA. 5×gDNase Mix can quickly remove residual genome from RNA at 42°C for 2 minutes, effectively avoiding genome interference on qPCR results. 5×RO-Easy™ Mix contains

Foregene Reverse Transcriptase specially developed by Foregene, which is a new type of reverse transcriptase with stronger RNA affinity. The optimized system makes the reverse transcription rate faster, and the first-strand cDNA synthesis can be completed in 15 minutes at 42°C. The reverse transcription system of this kit can also maintain good reverse transcriptase activity at 50°C, so it is more advantageous for complex RNA templates, and can easily transcribe RNA templates with high GC content and complex secondary structure.

Product characteristics

- Efficient removal of gDNA, which can remove gDNA from the template within 2 minutes.
- High-efficiency reverse transcription system can complete the first-strand cDNA synthesis in only 15 minutes.
- Complex templates: Templates with high GC content and complex secondary structures can also be reversed with high efficiency.
- High-sensitivity reverse transcription system, pg-level template can also obtain high-quality cDNA.
- The reverse transcription system has high thermal stability, the optimum reaction temperature is 42℃, and it still has good reverse transcription performance at 50℃.

Kit application

- Directly used for Real Time PCR quantitative analysis of gene expression.
- It can quickly and accurately analyze trace RNAs such as RNA viruses.
- Reverse transcription of RNA templates with high GC content or complex secondary structures.

Product quality control

Following the quality inspection system of Forgene kits (Foregene's total quality management system), each batch of the RT Easy™ II (with gDNase) kit must be strictly tested multiple times, and guarantee the reliability and stability of the kit's quality in each step.

Kit Contents

RT Easy™ II(With gDNase) Master Premix for first-strand cDNA synthesis for Real Time PCR		
Kit Contents	RT-01031	RT-01032
	25 rxns (20μl/reaction)	100 rxns (20μl/reaction)
5×gDNase Mix	50 μl	200 μl
5×RO-Easy™ Mix	100 μl	400 μl
RNase-Free ddH_2O	1.7 ml	1.7 ml
Instructions for Use	1	1

Transport and storage conditions

1. Transport conditions

The whole process should be low-temperature ice box transportation, to ensure that the kit is in a state of <4 ℃.

2. Storage conditions

Stored at -20 ℃. Store the product at -20℃ immediately after receipt. If the storage conditions are appropriate, the product will not degrade any performance during the 1-year validity period.

Kit contents information

- 5× gDNase Mix: gDNA remover, be sure to use this reagent according to the operating instructions before performing RT reaction (the internal glycerol content is high, and it may not freeze above -20°C, which is a normal phenomenon).
- 5×RO-Easy™ Mix: Contains Foregene Reverse Transcriptase, RNase Inhibitor, dNTPs, stabilizer, enhancer, optimizer and optimized ratio of reverse transcription primers (Random Primer, Oligo(dT)$_{18}$ Primer) specially developed by Foregene.

Precautions: **(please read carefully before using the kit)**

- It is recommended to use RNA extracted from fresh samples or stored at -80℃ (RNA should avoid repeated freezing and thawing).
- In 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; and disposable gloves and masks should be worn.
- Before use, put 5× gDNase Mix and 5×RO-Easy™ Mix on ice to melt completely, flick and mix well before use; the preparation of the system should be operated on an ice bath to improve the performance of the kit and the specificity of PCR amplification.
- 5×RO-Easy™ Mix has added reverse transcription primers with optimized ratio, no need to add any additional primers.
- For RNA templates with complex secondary structure, the reaction temperature can be increased to 50℃.

Amount of template RNA Template

RNA should preferably be extracted from fresh samples or stored at -80°C (repeated freezing and thawing of RNA should be avoided).

RT Easy™ II(With gDNase): (0.1pg-1μg total RNA or 0.01pg-0.1μg mRNA)/20μl system

Preparation before operation

It is strongly recommended that users read the instructions carefully before using this kit. . RT Easy™ II (With gDNase) kit is simple, convenient and fast to operate, and the instruction manual provides the correct use of the entire kit. Please prepare necessary experimental materials and equipment before use.

Experimental materials and equipment

- PCR machine or metal bath
- Micropipettes,RNase-Free pipette tips
- RNase-Free tube
- Ice bath

Self-provided reagents

- RNA template

Safety

- This product is for scientific research only, please do not use it for medicine, clinical medicine, food and cosmetics.
- Wear suitable lab coats, gloves, safety glasses, etc. when working with chemicals.

Operation guide

A: Preparation of materials and reagents

1. Prepare the RNA template (it is recommended to use Foregene Total RNA Isolation Kit series kits to extract and purify RNA), and related consumables and instruments.

   Note: Please make sure that RNA is not degraded.

2. Take out 5×gDNase Mix and 5×RO-Easy™ Mix, and place them on an ice bath to melt it. Flick the tube wall several times and mix well for later use. Take out RNase-Free ddH_2O after melting and place it on an ice bath for later use.

B:Reaction of gDNA removal

1. Prepare the reaction mixture on ice according to the following components.

In order to ensure the accuracy of the preparation of the reaction solution, when performing each reaction, the gDNA Mix should be prepared according to the number of reactions + 2, and then dispensed into each aliquot. The RNA sample was added last to each reaction tube. Refer to the following table 1 for system configuration:

Table 1: de-gDNA System Preparation

Content Items added in the system	Amount
5× gDNase Mix	2 μl
Template (RNA)	Xμl (Total RNA:<1μg / mRNA:<0.1μg)
RNase-Free ddH_2O	(8-X)μl
Total Volume	10μl

2. After the preparation of the system is completed, perform the de-gDNA reaction according to the reaction conditions in Table 2 below.

Table 2: de-gDNA Reaction Conditions

Steps	Temperature	Time	Content
1	42℃	2 min	de-gDNA reaction
2	4℃	N/A	After the reaction is completed, store at 4°C for use or -20°C

3. After the reaction, put it on ice and prepare the RT reaction system according to Table 3.

C: RT system preparation

1. Use the de-gDNA reaction solution obtained in step B as a template to prepare RT system, just add 5×RO-Easy™ Mix to the reaction tube of step B for the reaction. The specific RT reaction system preparation can refer to Table 3 below.

Table 3: RT System Formulation

Content of RT reaction mixture	Amount
All reaction solution in step B	10μl
5×RO-Easy™ Mix	4μl*
RNase-Free ddH_2O	(9-X)μl
Total Volume	20μl

*: Add 5× RO-Easy™ Mix directly to the reaction tube after the reaction in step B and add water to make up the system to proceed to the next reaction. The reverse transcription primer has already been added to 5×RO-Easy™ Mix, so there is no need to add it.

2.After the system is prepared, mix gently and briefly centrifuge to perform RT reaction according to the reaction conditions in Table 4 below.

Table 4: RT Reaction Conditions

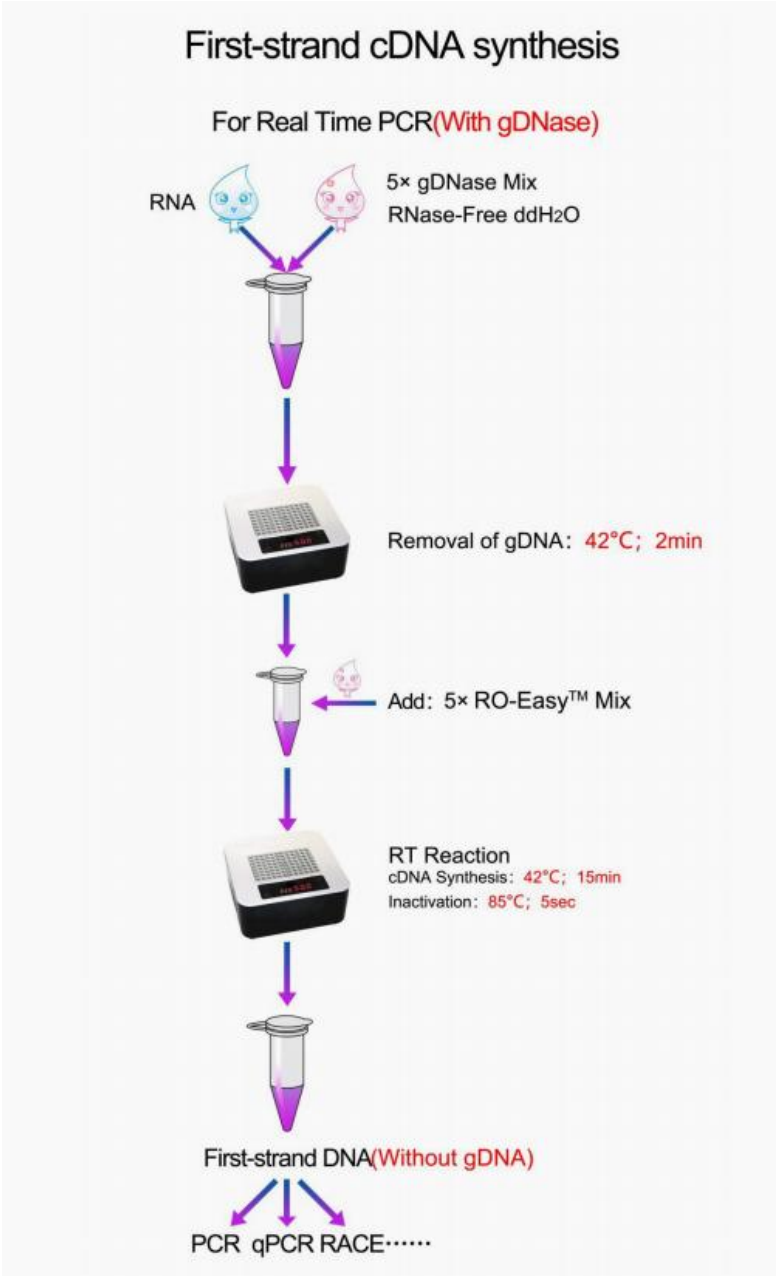
Steps	Temperature	Time	Content
1	42℃	15 min	Inactivation of gDNase and cDNA synthesis
2	85℃	5 sec	Inactivation of reverse transcriptase
3	4℃	N/A	After the reaction is completed, store at 4°C for use or -20°C

Note: The above procedure is for reference only, and the actual reaction conditions vary depending on the structural conditions of templates, primers, etc. For RNA templates with complex secondary structures, it is recommended to use 50°C in the first step, and 42°C for other RNA templates.

3. After the reaction is completed, put it on ice and use it directly for Real Time PCR. Please store it at -20°C for long-term storage.

Note: The obtained RT reaction solution is added to the next Real Time PCR reaction system, and the amount added should not exceed 1/10 (V/V) of the Real Time PCR reaction volume.

Operation Diagram



Problem analysis guide

The following is an analysis of the problems that may be encountered in the use of the RT Easy series kits, and hope it will be helpful to your experiments. In addition, we will provide specialized technical support to help you with other experimental or technical issues other than the operating instructions. If you have any needs, please contact us: 86-28-83360257 or E-mail: Tech@foregene.com.

Non-specific amplification

1. Unreasonable primer design
   Suggestion: Design the primers according to the principle of primer design.
2. Genomic residues.
   Suggestion: Make sure the reaction temperature for the first step of gDNA removal is 42°C, and the reaction time can also be extended to 5min.

No amplified signal after RT-PCR

1. RNA is degraded.
   Suggestion: The materials for extracting RNA should be as fresh as possible, using high-quality and high-purity RNA.
2. RNA contains inhibitors.
   Suggestion: Reverse transcription inhibitors generally include SDS, guanidine salt, EDTA, etc. It is recommended to wash the RNA precipitate with 70% ethanol to remove the inhibitor.
3. The problem of primer design.
   Suggestion: According to the primer design principle, redesign the primer for inspection.

The target band appears in the blank control

1. The operating tools or reagents are contaminated.
   Suggestion: All reagents or equipment in the experiment should be autoclaved. Be careful and gentle during operation to prevent the DNA samples from being sucked into the pipette or spilled out of the centrifuge tube.
2. Contamination occurred during the preparation of the PCR reaction system.
   Suggestion: Take necessary protective measures during operation, such as wearing latex gloves and using pipette tips with filter elements. Use real time PCR Mix with anti-pollution system.
3. The primers are degraded.
   Suggestion: Use SDS-PAGE electrophoresis to detect whether the primers are degraded, and replace with new primers for fluorescence detection experiments.

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