FOREGENE

RT Easy™ I

Master Premix for first-strand cDNA synthesis

Cat.No.RT-01011/01012

Fast and highly sensitive reverse transcription system for generating first-strand cDNA using pg-level RNA

For research use only

Store at -20℃

Contents

Product Introduction

FOREGENE's 2× RT Easy™ Mix can synthesize the first-strand cDNA at a temperature as high as 50°C, 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 transcriptases, resulting in unsatisfactory reverse transcription effects or low reverse transcription efficiency. FOREGENE 2× RT Easy™ Mix shows extremely high tolerance to alcohol and guanidine salt, the highest tolerance to alcohol in RNA samples is 45%, and the highest tolerance to guanidine salt is 750mM. Even if the RNA is not pure, the reverse transcription reaction can be performed with FORREGENE 2×RT Mix. The unique reaction system makes the RT reaction easier, faster and more efficient, and the synthesis of the first strand cDNA can be completed in 25 minutes.

This product can be used in conjunction with Foregene's fluorescent quantitative product Real Time PCR Easy™ to obtain high-quality experimental results.

Features

- Efficient reverse transcription system, it only takes 25 minutes to complete the synthesis of the first strand cDNA.
- High-sensitivity reverse transcription system, pg-level templates can also get high-quality cDNA.
- The reverse transcription system has high thermal stability, the reaction temperature of the system can be as high as 50 ℃, and it has good reverse transcription performance.
- Even impure RNA samples (alcohol up to 45%, guanidine salt up to 750mM) can be subjected to reverse transcription reaction.

Kit application

- Conventional RT-PCR
- Synthesis of cDNA for cloning and expression research
- Analysis of the primer extension method of the transcription initiation site
- RACE (rapid amplification of cDNA ends)
- Linear RNA amplification
- Chip mark

PRODUCT QUALITY CONTROL

In accordance with FOREGENE's Total Quality Management System (FOREGENE's Total Quality Management System), each batch of RT Easy™ I kits are strictly tested multiple times to ensure the reliability and stability of the quality of each batch of kits.

Kit Contents

RT Easy™ I Master Premix for first-strand cDNA synthesis		
Kit contents	RT-01011	RT-01012
	25 times (20μl system)	100 times (20μl system)
2× RT Easy™ Mix	0.25ml	1ml
Random Primer (50μM)	50μl	200μl
Oligo(dT)18 Primer (50μM)	50μl	200μl
RNase-Free ddH_2O	1.7ml	1.7ml
Instructions Manual	1 piece	1 piece

Transport and storage conditions

1.Transportation conditions

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

2. Storage conditions

RT Easy™ I is stored at -20°C. Store the product in a constant temperature refrigerator at -20°C immediately after receipt. If the storage conditions are appropriate, the product will not degrade any performance during the 1-year validity period.

Kit component information

- 2× RT Easy™ Mix: Foregene Reverse Transcriptase, RNase Inhibitor, dNTPs, reaction buffer, optimizer and stabilizer, etc.
- Random Primer (50μM): 6 oligonucleotide random primers with a concentration of 50μM.
- Oligo(dT)18 Primer(50μM): a primer with 18 dT,the concentration is 50μM

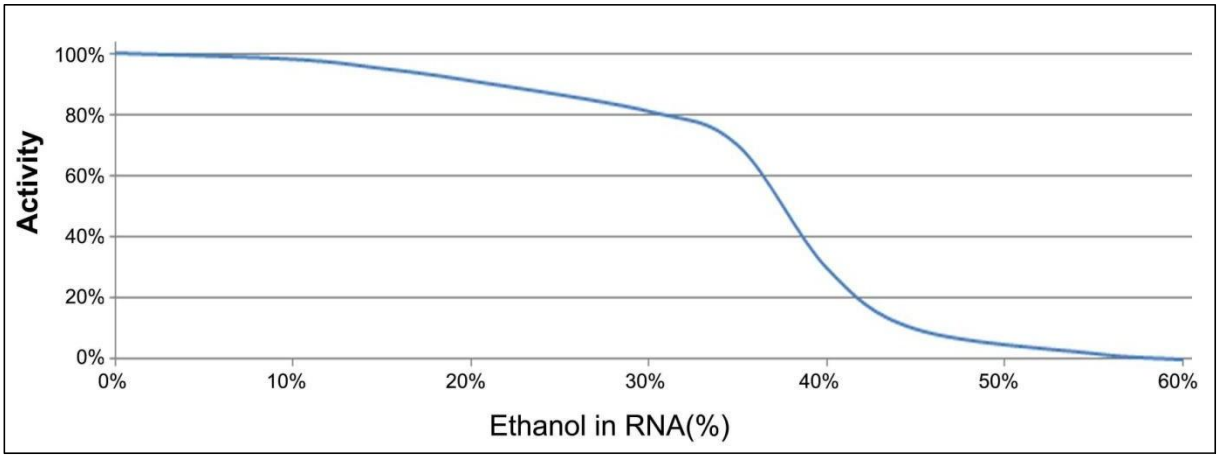
Precautions: (Please read the precautions carefully before using the kit)

- For templates, 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 the 2×RT Easy™ Mix on ice to completely melt, 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.
- 2× RT Easy™ Mix does not add reverse transcription primers, you need to add appropriate concentrations of Random Primer or Oligo(dT)18 Primer or specific primers according to the experimental requirements.

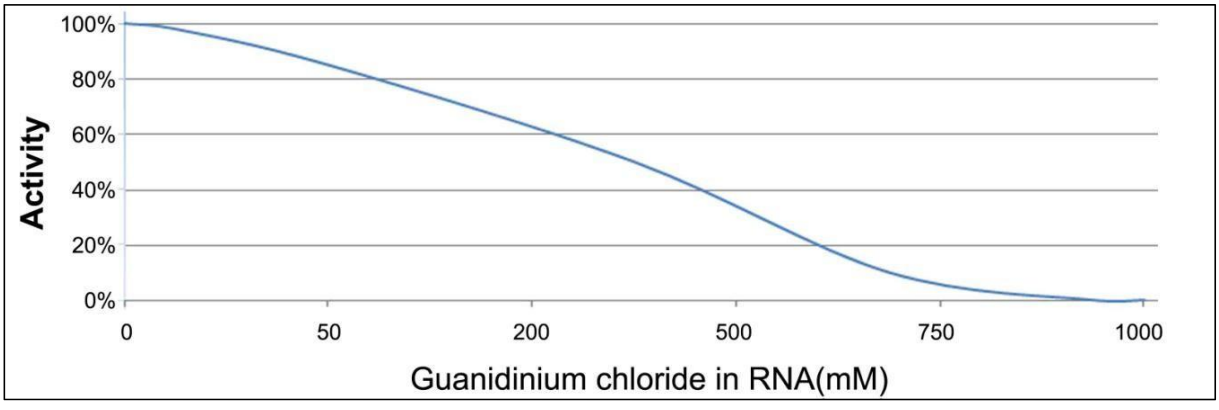
RT Mix tolerance

After testing and analysis, 2 × RT Easy™ Mix shows extremely high tolerance to alcohol and guanidine salts. The RNA purified in the laboratory often has alcohol and guanidine salt residues, which have a strong inhibitory effect on reverse transcription, resulting in unsatisfactory reverse transcription effect or low reverse transcription efficiency. The tolerance of Foregene RT Easy series products to alcohol and guanidine salts makes reverse transcription easier and more efficient.

Alcohol tolerance analysis



Guanidine salt tolerance analysis



Preparations before operation

It is strongly recommended that users read the instructions carefully before using this kit. The RT Easy series kits are simple, convenient and fast to operate. The instructions provide the correct use of the entire kit. Please prepare necessary experimental materials and equipment before use.

Amount of reverse transcription primer

RT Easy™ I needs to add reverse transcription primers. Please add the appropriate concentration of primers according to the experimental needs according to the following table.

Primer name	Applicable Scope	Primer Amount
Random Primer	It is suitable for long RNA or RNA with secondary structure. This primer can be used for reverse transcription reaction of all RNA including rRNA, mRNA, tRNA, etc.	50 pmol
Oligo(dT)18 Primer	Suitable for RNA with Poly (A) tail Note: Prokaryotic RNA, eukaryotic rRNA, tRNA, and certain types of eukaryotic mRNA do not have Poly (A).	50 pmol
Specific downstream primer	Must be complementary to the template sequence, need to know the target sequence	2 pmol

Amount of template RNA

For template RNA, it is best to use RNA extracted from fresh samples or stored at -80°C (RNA should avoid repeated freezing and thawing).

RT Easy™ I： (0.1pg-5μg total RNA or 0.01pg-0.5μg mRNA)/20μl system.

Experimental materials and equipment

- PCR thermal cycler or metal bath
- Micro pipette, RNase-Free tip
- RNase-Free tube
- Ice bath

Bring your own reagents

- RNA template
- Gene specific primers (if necessary)

Safety

- This product is for scientific research use only, please do not use it in medicine, clinical medicine, food and cosmetics.
- Wear suitable laboratory clothes, gloves, protective glasses, etc. when using chemicals.

Operation guide

A: Preparation of materials and reagents

1. Prepare the prepared 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: As the template RNA, please make sure that the RNA is not degraded or use newly extracted RNA.

2. Take out 2× RT Easy™ Mix and place it on an ice bath to melt it naturally. Flick the tube wall several times and mix well for later use; Take out RNase-Free ddH_2O and melt it and place it on an ice bath for later use; Take out Random Primer or Oligo(dT)18 Primer or specific primer melted as needed and put on ice for later use.

B: RT system preparation

2×RT 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. When using, just take half the volume of the reaction system (for example, if the reaction system is 20μl, take 10μl 2×RT Easy™ Mix solution), add RNA template and reverse transcription primers, and add RNase-Free ddH_2O to make up the volume 20μl. The specific RT reaction system preparation can refer to Table 1 below.

Table 1: RT system preparation

RT System Added contents	Amount
2× RT Easy™ Mix	10μl
Random Primer(50μM)	1μl*
or Oligo(dT)18 Primer(50μM)	1μl*
or Specific Primer(2μM)	1μl*
Template(RNA)	Xμl (Total RNA:<5μg / mRNA:<0.5μg)
RNase-FreeddH_2O	(9-X)μl
Total Volume	20μl

*: Please refer to "Reverse Transcription Primer Amount" on page 7 for the amount of primer.

C: RT reaction program setting

1. After preparing the RT system according 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 cap, and place it in Ready to use on ice box).

2. Refer to the RT reaction program settings (Table 2) to set the temperature and time of the reaction.

Note: In order to ensure the activity of 2× RT Easy™ Mix and improve its amplification efficiency, it is best to prepare the RT reaction system after the metal bath reaches the appropriate reaction temperature, so that the reaction program can be entered immediately after the system is prepared. The RT reaction can also be performed on a thermal cycler.

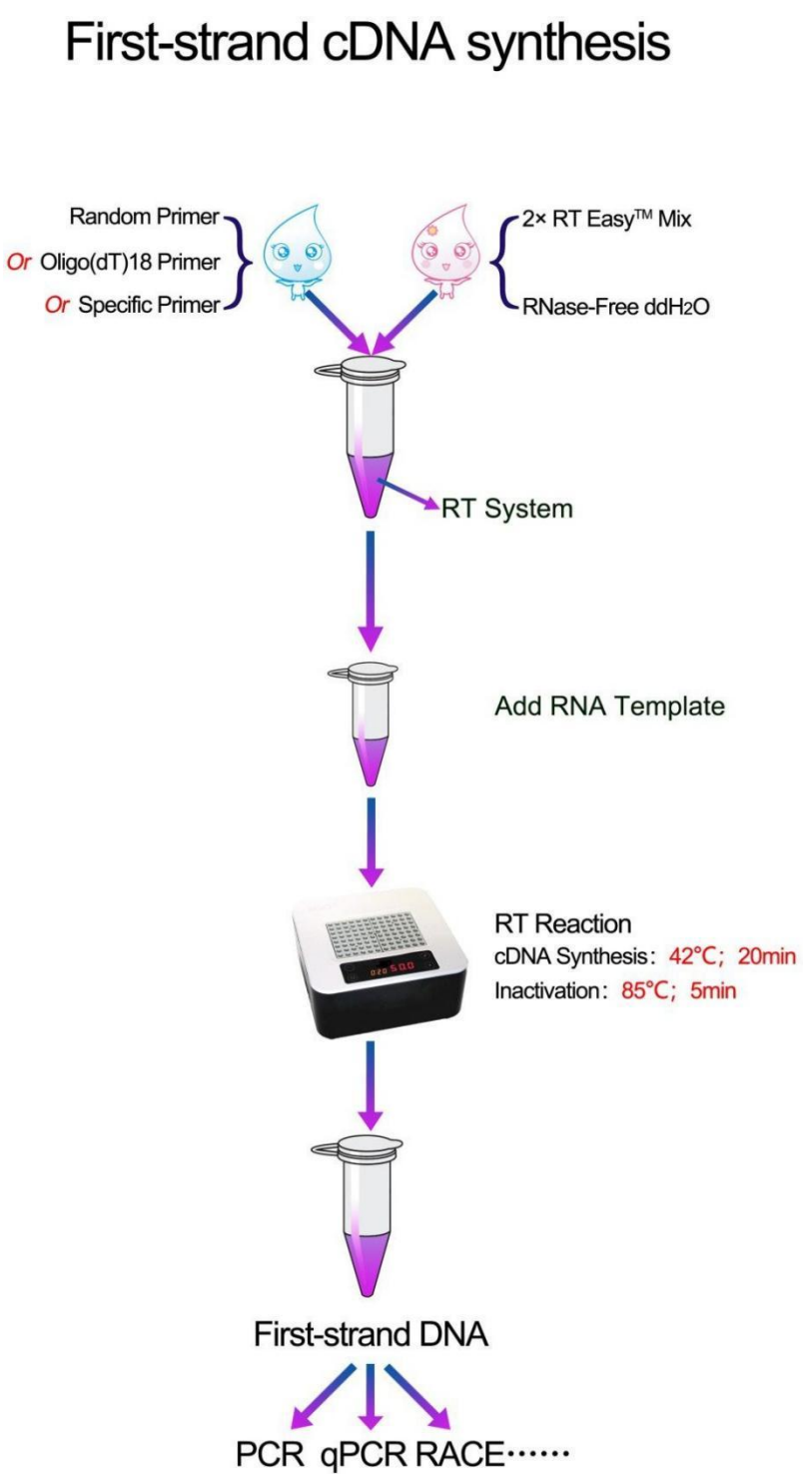
3. The reaction product can be directly used in subsequent tests, or stored at -20°C for up to a week. Long-term storage is recommended to be at -80℃, avoid repeated freezing and thawing.

Table 2: RT reaction program settings

Step	Temperature	Time	Contents
1	42℃	20 min	Reverse Transcription
2	85℃	5min	Inactivation

Note: Generally, 42℃ is recommended for the first step reaction temperature; for RNA templates with complex secondary structure, the first step reaction temperature is recommended to use 50℃ or 65℃ . When using Random Primer, the reaction should be preheated at 25℃ for 10 minutes before the first step of the reaction. The above procedure is for reference only. The actual reaction conditions vary depending on the structural conditions of the template, primers, etc.

Quick operation diagram



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