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FOREGENE

Lnc-RT Hero™ I(With gDNase)

Super Premix for first-strand cDNA synthesis from lncRNA

Cat.No.RTL-09098/09099

Fast and highly sensitive reverse transcription system for generating first-strand cDNA from lncRNA

For research use only

Store at -20℃

Product description

Lnc-RT Hero™ I(With gDNase) is a reverse transcription system specially developed for lncRNA to rapidly remove contamination from genomic DNA. 5×gDNase Mix can quickly remove residual genome in RNA at 42°C for 2 minutes, effectively avoiding genome interference on qPCR results.

5×L-RT Hero™ Mix contains Foregene LncRNA Reverse Transcriptase specially developed by Foregene, which is a new type of reverse transcriptase specially designed for long RNA complex templates such as lncRNA, with stronger RNA affinity and higher reverse transcriptase recording efficiency. The optimized system makes the reverse transcription rate faster, and can easily transcribe RNA templates with high GC content and complex secondary structure. The first-strand cDNA synthesis can be completed in 15 minutes at 42°C.

Features

- Efficient removal of gDNA, which can remove gDNA in the template within 2min.
- The lncRNA can be efficiently reverse transcribed, and the Ct value of the reverse transcription product is lower than that of the conventional reverse transcription system when performing qPCR.
- Efficient reverse transcription system, it only takes 15 minutes to complete the first-strand cDNA synthesis.
- Complex templates: Templates with high GC content and complex secondary structures can also be reversed with high efficiency.
- High-sensitivity reverse transcription system, high-quality cDNA can also be obtained from pg-level templates.
- 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

- lncRNA relative quantitative analysis.
- lncRNA absolute quantitative analysis.
- Can quickly and accurately analyze microRNA such as RNA virus.
- Reverse transcription of RNA templates with high GC content or with complex secondary structures.

Product Quality Control

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

Kit components

Lnc-RT Hero™ II(With gDNase) Super Premix for first-strand cDNA synthesis for lncRNA with gDNase		
Kit component	RTL-09098	RTL-09099
	25T (20μl system)	50T (20μl system)
5× gDNase Mix	50μl	50μl ×2
5× L-RT Hero™ Mix	100μl	100μl ×2
RNase-Free ddH_2O	1.7ml	1.7ml
Manual	1	1

Shipping and Storage Conditions

1. Shipping Conditions

The whole process is transported on dry ice to ensure that the kit is in a state of <-20 °C.

2. Storage conditions

The kit was stored at -20°C. Store the product in a -20°C constant temperature refrigerator immediately upon receipt. If stored properly, the product will not degrade any performance during the 1-year validity period.

Kits component information

- 5×gDNase Mix: gDNA removing agent, it is necessary to use the reagent in accordance with the operating instructions before the RT reaction is performed (the internal glycerol content may not be frozen, and it may not be frozen, which belongs to normal phenomena).
- 5×L-RT HERO™ MIX: Contains Foregene Lncrna Reverse Transcriptase, RNase Inhibitor, DNTPS, Stabilifer, Enhancer, Optimizer, Oligo ($DT_{)18}$ primer).

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

- Template recommends using fresh samples or RNA preserved under -80 ° C (RNA should avoid repeated freeze-thaw, ensuring RNA integrity, no degradation).
- In order to avoid RNASE pollution, the experimental operation is carried out in RNase-Free space; the gun head and PCR centrifuge must be guaranteed to be RNASE-Free; and wear disposable gloves and masks.
- Before using, 5 × GDNase Mix and 5 × L-RT HERO™ MIX are placed on ice make it completely melted, and the light bullet is mixed; the system is formulated, please operate on an ice bath to improve the performance of the kit, improve the performance of the kit PCR amplification specificity.
- 5 × L-RT HERO™ MIX has added an optimized ratio reverse transcription primer without adding any primers to additional additional primers.
- For the complex RNA template of the secondary structure, the reaction temperature can be increased to 50 ° C

Template RNA requirements

It is best to use RNA extracted from fresh samples or preserved at -80 °C.

- Template integrity: good integrity, no degradation.
- Template purity: IONS, proteins, EDTA, ethanol, phenols and other journals contained in RNA will affect reverse transcriptase activity and ultimately affect reverse transcription effects.

Template RNA dosage

- For lncRNA: 10ng-1 μg total RNA/20μl system.
- For general RNA: 0.1pg-1 μg total RNA or 0.01pg-0.1 μg mRNA/20 μl system.

Preparation before operation

It is strongly recommended that users read the instructions carefully before using this kit. The Lnc-RT Hero™ I (With gDNase) kit is simple, convenient and fast to operate, and the instructions provide the correct use of the entire kit. Please prepare necessary experimental materials and equipment before use.

Experimental Materials and Equipment

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

Self-provided reagents

- RNA template

Safety

- This product is for scientific research use 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 Steps

A: Preparation of materials and reagents

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

Note: As template RNA, make sure the RNA is not degraded or freshly extracted.

2. Take out 5×gDNase Mix and 5×L-RT Hero™ Mix and put them on the ice bath, let them melt naturally, and gently knead and mix until use; take out RNase-Free ddH_2O to melt and put it on the ice bath for use.

B: De-gDNA reaction

1. Prepare the reaction mixture on ice according to the following ingredients. In order to ensure the accuracy of the reaction solution preparation, when performing each reaction, the gDNA Mix should be prepared according to the number of reactions + 2, and then dispensed into each reaction tube. , the RNA sample is added last. Refer to Table 1 for system configuration:

Table 1: De-gDNA system formulation

System additions	Amount
5× gDNase Mix	2μl
Template(RNA)	Xμl (lncRNA/Total RNA:<1μg / mRNA:<0.1μg)
RNase-Free ddH_2O	(8-X)μl
Total Volume	10μl

2. After the preparation of the system, the de-gDNA reaction was carried out according to the reaction conditions in Table 2 below.

Table 2: De-gDNA Reaction Conditions

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

2. After the reaction was completed, 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 for RT system preparation, just add 5×L-RT Hero™ Mix to the reaction tube in step B for the reaction. The specific RT reaction system preparation can refer to Table 3 below.

Table 3: RT system formulation

RT system additions	Addition
All Reaction solution in step B	10μl
5× L-RT Hero™ Mix	4μl *
RNase-Free ddH_2O	6μl
Total Volume	20μl

*: Add 5×L-RT Hero™ 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 been added to 5×L-RT Hero™ Mix, and there is no need to add it.

1. After the preparation of the system, mix gently and briefly centrifuge, and then perform RT reaction according to the reaction conditions in Table 4 below.

Table 4: RT Reaction Conditions

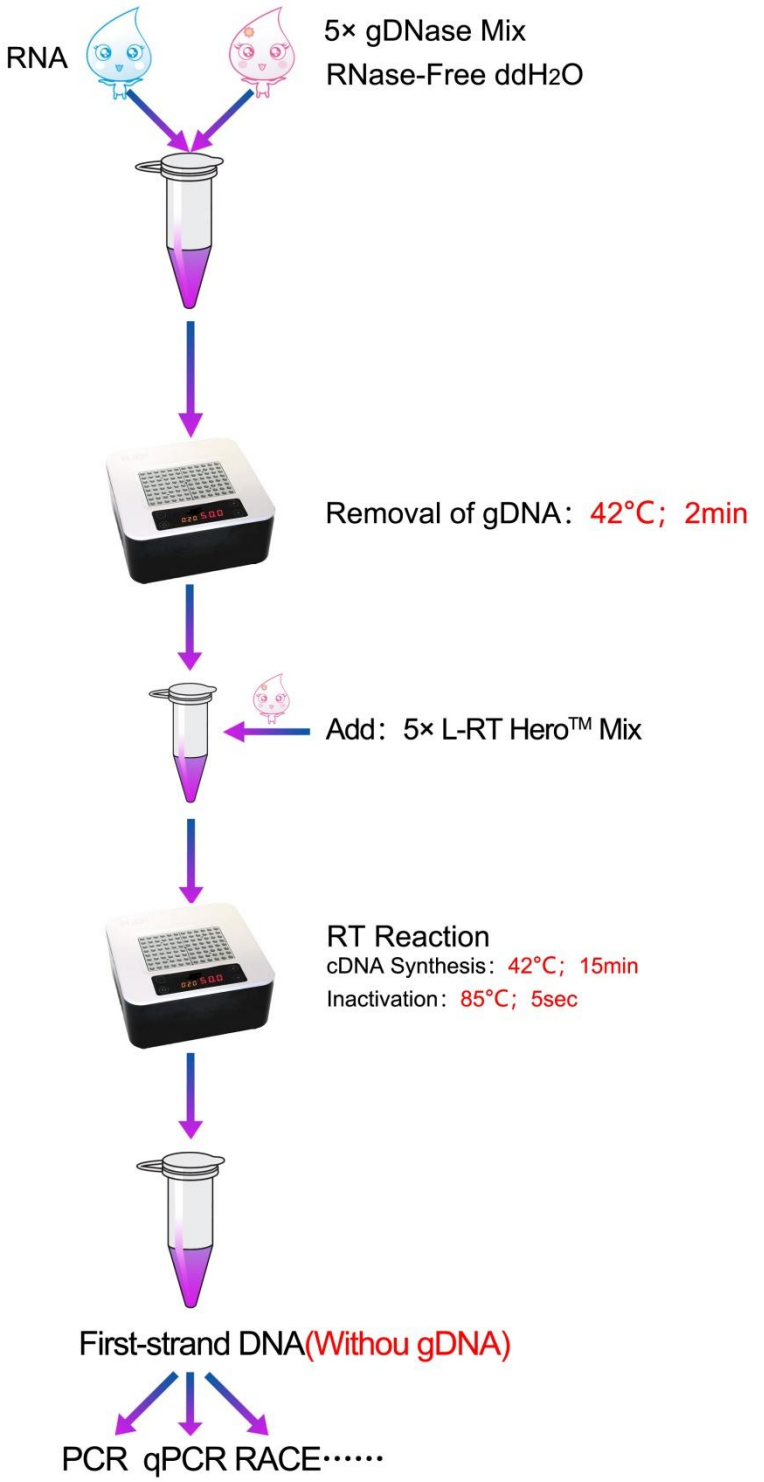
Steps	Temperature	Time	Contents
1	42℃	15min	Inactivation of gDNase and cDNA synthesis
2	85℃	5sec	Inactive reverse transcriptase
3	4℃	N/A	After the reaction is completed, store at 4°C for use or -20°C

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.

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

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

Quick operation diagram



Problem Analysis Guide

The following is an analysis of the problems that may be encountered in the use of Lnc-RT HeroTM I (With gDNase) series kits, hoping to be helpful to your experiments. In addition, we have dedicated technical support to help you with other experimental or technical problems beyond the operating instructions and questions. If you have any needs, please contact us: 028-83361257 or E-mail: Tech@foregene.com.

Non-specific amplification

1. Unreasonable primer design

   Recommendation: Design primers according to primer design principles.

2. Genome residues.

   Suggestion: Make sure that the first step of gDNA removal reaction temperature is 42°C, and the reaction time can also be extended to 5min.

No amplification signal by RT-qPCR

1. RNA is degraded.

   Recommendation: The material for RNA extraction should be as fresh as possible, and high-quality and high-purity RNA should be used.

2. RNA contains inhibitors.

   Recommendation: Reverse transcription inhibitors generally include SDS, guanidine salts, EDTA, etc. It is recommended to wash the RNA precipitate with 70% ethanol to remove the inhibitors.

3. Primer design issues.

   Recommendation: According to the primer design principle, redesign the primers for inspection.

The target band appears in the blank control

1. Contamination of operating tools or reagents.

   Recommendation: All reagents or equipment for the experiment should be autoclaved. Be careful and gentle when handling to prevent 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.

Recommendation: Take necessary precautions when handling, such as: wearing latex gloves, using filter tips. Use Real Time PCR Mix in a contamination-proof 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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