For research use only Version Number：1.0-2110

Direct RT-qPCR Kit III

Kit contents (20 μL reaction system)	IM-05111-03 500 T	IM-05112-03 50,000 T
Foreasy Reverse Transcriptase (200 U/μL)	50 μL ×1	1 mL ×5
2× Direct qPCR Mix-Taqman	1 mL ×5	500 mL ×1

Note: ROX Reference Dye can be provided with the kit if necessary.

Product introduction

Direct RT-qPCR Kit provides separately packaged RT-qPCR buffer(with Foreasy HS Taq DNA polymerase mixed) and high efficient Foreasy Reverse Transcriptase(MMLV), making it convenient for the creating of back-end kits and system adjustment，within simple verification.

Direct RT-qPCR Kit, using Foreasy Reverse Transcriptase and Foreasy HS Taq DNA Polymerase developing by Foregene, with unique reaction buffer, makes this kit with strong resistance and compatibility, and it can directly test reaction use Foregene Lysis system as template. This kit can apply for COVID-19 nucleic acid detection kit and other pathogenic bacteria nucleic acid detection kit development.

This kit can directly be the component of the IVD product, using easily and fast. It only needs easy verification, without developing again.

Storage Condition

Storage condition: The kit should be stored at -20 ± 5℃. It should immediately be stored in -20℃ thermostatic refrigerator. The validity period is 2 years if it be stored in suitable condition.

Component of Kit

- 2× Direct RT-qPCR Buffer：dNTPs, Mg^{2+}, stabilizer, reinforcing agent and optimizing agent with optimized ratio.
- Direct Enzyme：Foreasy Reverse Transcriptase(M-MLV)、Foreasy HS Taq DNA Polymerase、Foreasy RNase Inhibitor and protectant buffer.

Note

- Avoid repeated freeze-thaw cycles, otherwise it would result in the kit with less efficient or out of operation.
- It should be operated in RNase-Free condition to avoid RNase pollution. All of the pipette micro tips, PCR tubes used should be RNase-Free. The operators should wear disposable gloves and masks.
- This kit needs specific primers and probes, please use specific primers and probes of the amplified gene according reality experiment.
- Before reagent preparation, the 2× Direct RT-qPCR Buffer should be thawed on ice and mixed gently. The reagent preparation should be operated on ice to enhance the efficiency of the kit and improve the specificity of PCR amplification.

RNA Dosage

- (1pg-100ng total RNA) / 20 μL reaction system.
- Non-purified templates, such as sample release agent Lysis products, needs to optimize the amount of template added selfly. Foregene Lysis system is recommended.

Operating Steps

A: Prepare of template and reagent

1. Prepare prepared RNA template (It is recommended to use Foregene Total RNA Isolation Kit series kit to extract and purify RNA template) or sample cracking product (It is recommended to use Foregene Lysis system), specific primers(10 μM) and other relevant consumables, instrument.
2. Put the 2× Direct RT-qPCR Buffer, RNase-Free ddH_2O and 20× ROX Reference Dye(if it's needed) in the box with ice, making these melt naturally, and mixed gently.

B: Prepare of RT-qPCR system

Get half the volume of reaction buffer of reaction system (for example, if the volume of the system is 20 μL, it needs to get 10 μL 2× Direct RT-qPCR Buffer). For corresponding amount of enzyme,we suggest that M-MLV： 10-30 U/20 μL reaction system,you should test and adjust it), add RNA template, specific primers and probes, and add RNase-Free ddH_2O to 20 μL. The specific preparation of RT-qPCR reaction system can be referred to the following table 1.

Table 1 : Prepare of RT-qPCR system

Component	Volume	Final concentration
2× Direct RT-qPCR Buffer(wiht Taq DNA)	10 μL	1×
Foreasy Reverse Transcriptase(MMLV)	10-30 U	
Forward Primer(10 μM)	0.8 μL	50-900nM
Reverse Primer(10 μM)	0.8 μL	50-900nM
Probe(4 μM)	1 μL	200nM
Template(RNA or Lysate)	X μL	
20× ROX Reference Dye	-	1*
RNase-FreeddH_2O	(6.4-X) μL	
Total Volume	20 μL	

Note: Forward Primer and Reverse Primer are specific primers of target gene. The system of the qPCR could be adjusted according to practical experimental and PCR models. We recommend 400nM for the final concentration of most primers. Adjust the volume of specific primers and probes according to the prepared concentration and the recommended final concentration, please.

1*: Select suitable final concentration of ROX Reference Dye according to different quantitative PCR instruments. The optimal ROX Reference Dye concentration of common quantitative PCR instruments is shown in the table following:

Quantitative PCR instruments	Final concentration of ROX Reference Dye
ABI PRISM 7000/7300/7700/7900HT/Step One, etc.	1× (for example, add 1μl 20×ROX Reference Dye in 20 μL reaction system)
ABI 7500, 7500 Fast, Stratagene Mx3000P, Mx3005P and Mx4000, etc.	0.5× (for example, add 0.5μl 20×ROX Reference Dye in 20 μL reaction system)
Roche PCR instrument, Bio-Rad PCR instrument, Eppendorf quantitative PCR instrument, etc.	Without ROX Reference Dye

C: Set the RT-qPCR reaction program

1. After the RT-qPCR system is prepared according to the above table, the liquid scattered on the pipe wall is collected by instantaneous centrifugation and placed on the ice box for use.
2. Set reaction condition like temperature and time according to the RT-qPCR reaction program Settings (Table 2).
   Note: In order to ensure the activity of RT-qPCR reaction and improve its amplification efficiency, it's better to prepare the RT-qPCR reaction system after setting up the PCR instrument program so that the system can enter the reaction procedure immediately after the preparation of the system.
3. In order to obtain the best PCR effect, gradient PCR is optional to optimize reaction conditions for different templates and different primers.
   Note: The extend the range of temperature of Foreasy HS Taq DNA Polymerase provided in this kit are 60-72 ℃, and the best extend temperature is 72 ℃.

Table 2 : RT-qPCR reaction program setting (for example: two-step method)

Step	Temperature	Time	Cycles	Content
1	50℃	15 min 1*	1	Reverse transcription
2	95℃	1 min	1	Pre-denaturation
3	95℃	10 sec	40-45	Denaturation
	60℃	30 sec 2*		Anneal/Extension

1*: The reverse transcription time can be adjusted according to the needs of the experiment. For ordinary endogenous genes, such as β-Actin, it only takes 10 minutes. Detection of specific expression genes can be appropriate to extend the reverse transcription time.

2*: The time of anneal or extension is decided by the length of the amplified DNA fragment, and the amplification rate of Foreasy HS Taq DNA Polymerase was 2 kb/min.

Note: The PCR reaction condition depends on the structural conditions of templates and primers. In practice, it is necessary to design the best reaction conditions, including annealing temperature and extension time, according to the size of the target fragment, base sequence of the amplified fragment and GC content and length of the primer.