FOREGENE
EXPERT IN DIRECT PCR DETECTION

QuickEasy™ Real Time PCR Kit-Taqman

For qPCR using cDNA, purified DNA

For research use only

Store at -20℃

Contents

Product Description

The 2× QuickEasy™ Real PCR Mix-Taqman provided by the QuickEasy™ Real Time PCR Kit-Taqman kit is a new generation master mix system designed for Real Time PCR amplification reaction using specific fluorescent probes. Its core Foregene HS Taq DNA Polymerase is based on Antibody modification, used in conjunction with Foregene further optimized PCR system, has higher specificity and amplification efficiency. Compared with ordinary PCR mix, it has stronger amplification ability and lower mismatch rate. It can be used in fluorescence quantitative PCR reaction to reduce non-specific amplification and improve the accuracy of PCR. It can greatly improve product specificity and reaction sensitivity. At the same time, ROX was provided as an internal reference dye.

The QuickEasy™ Real Time PCR Kit-Taqman kit has a broad scope of application to the amount of template, and can obtain a good standard curve in different quantitative regions, and can accurately quantify and detect target genes with good repeatability and high confidence.

Product Features

- The unique PCR optimization system makes 2×QuickEasy™ Real PCR Mix-Taqman more compatible.
- Hot-start Foregene HS Taq Polymerase has higher amplification efficiency, higher amplification sensitivity, and higher amplification specificity.
- 2× QuickEasy™ Real PCR Mix -Taqman uses a unique system optimized by Foregene to improve the sensitivity and specificity of sequence-specific probe detection, which can be used for genotyping and copy number variation determination and obtain accurate results.
- This product comes with ROX internal reference dye, which can be used to eliminate signal background and signal error between wells, which is convenient for customers to use in different types of quantitative PCR instruments.

Kit Application

- Fluorescence quantitative PCR for template quantitative analysis;
- Conventional PCR amplification;
- Can be used for allele detection.

Product Quality Control

According to FOREGENE's Total Quality Management System, each batch of QuickEasy™ Real Time PCR Kit-Taqman is strictly tested multiple times to ensure the reliability and stability of the quality of each batch of kits .

Kit contents

QuickEasy™ Real Time PCR Kit-Taqman (20μl system)				
Kit contents	QP-01121	QP-01122	QP-01123	QP-01124
	200 Preps	500 Preps	1000 Preps	2000 Preps
2× QuickEasy™ Real PCR Mix-Taqman	1 mL × 2	1.7 mL × 3	1.7 mL × 6	1.7 mL × 12
20× ROX Reference Dye	200 μL	0.5 mL	1 mL	1 mL × 2
DNase-Free ddH_2O	1.7 mL	1.7 mL × 2	10 mL	20 mL
IFU	1piece	1piece	1piece	1piece

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

The kit should be stored at -20°C in the dark; if it is used frequently, it can also be stored at 4°C

for a short period of time (use up within 10 days).

Kit component information

- 2× QuickEasy™ Real PCR Mix-Taqman: It contains the hot-start Taq DNA Polymerase specially modified by Foregene, $MgCl_2$, dNTPs with optimized ratio, reaction buffer, PCR reaction enhancer, optimizer and stabilizer, etc. For PCR reaction, just add the appropriate lysis mix, primers, DNase-ddH_2O to 2× QuickEasy™ Real PCR Mix-Taqman and it can be used for PCR reaction.
- ROX Reference Dye: Generally used on Real Time PCR amplifiers of ABI, Stratagene and other companies to adjust the difference between PCR tubes and tubes caused by PCR sampling errors. The concentration of ROX Reference Dye required by different instruments is different, and users can add it according to the recommended concentration of the instrument.
- DNase-Free ddH_2O: Ultrapure water for PCR reactions.

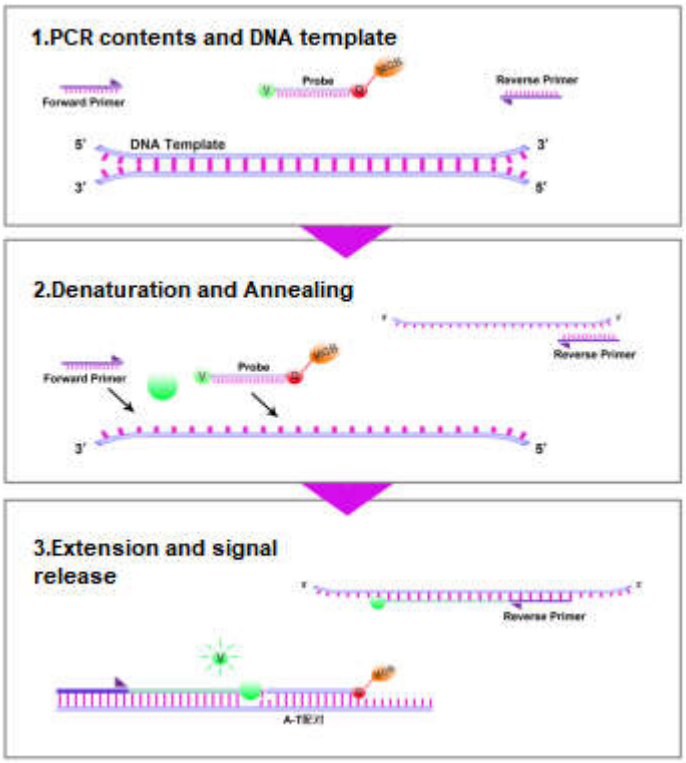
Precautions: **(Please read the precautions carefully before using the kit)**

- Store 2× QuickEasy™ Real PCR Mix-Taqman at -20°C. Repeated freezing and thawing should be avoided, otherwise the PCR efficiency will be affected.
- When using, please invert the 2× QuickEasy™ Real PCR Mix-Taqman upside down and mix gently to avoid foaming, and centrifuge briefly before use. If the reagents are not mixed, their reaction performance will be reduced. Never use a shaker to mix.
- Please use new (non-polluting) pipette tips, PCR tubes, etc. for the preparation and dispensing of the reaction solution to avoid contamination as much as possible.

Kit principle

During PCR amplification, a specific fluorescent probe is added along with a pair of primers. The probe is an oligonucleotide, and the two ends are respectively labeled with a reporter fluorescent group and a quenching fluorescent group. When the probe is intact, the fluorescent signal emitted by the reporter group is absorbed by the quencher group; during PCR amplification, the 5'-3' exonuclease activity of Taq enzyme cleaves and degrades the probe, making the reporter

fluorophore and the quencher group. The fluorescent group is separated, and the fluorescence monitoring system can receive the fluorescent signal, that is, each time a DNA chain is amplified, a fluorescent molecule is formed, and the accumulation of the fluorescent signal is completely synchronized with the formation of the PCR product. Quantitative analysis was then performed by monitoring the accumulation of fluorescent signals throughout the PCR process in real time and comparing with the standard curve.



Precautions before operation

Please read the instructions carefully before using this kit.

QuickEasy™ Real Time PCR Kit-Taqman is simple, convenient and fast to operate, and the instruction manual provides complete information and correct usage of the entire kit. Please prepare necessary experimental materials and equipment before use.

Experimental materials and equipment

- 0.2 mL sterile PCR tube.

- Fluorescence quantitative PCR amplifier, micropipette, ice bath.
- Self-prepared DNA template, PCR primers, Probe.

Safety

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

Operations Guide

A: Real Time PCR system preparation

1. Take out 2× QuickEasy™ Real PCR Mix-Taqman, 20× ROX Reference Dye, primers, Probe, etc. and put them in the ice box, and let them melt naturally. After thawing, mix the reagents upside down and centrifuge briefly to collect the liquid scattered on the tube wall and lid.

Note: 2 × QuickEasy™ Real PCR Mix-Taqman will become turbid when placed at room temperature or held in your hand for a long time. You can put it on ice for 2-5min. When the solution is clear, invert and mix 3-5 times before use.

2. Add an appropriate amount of DNA template, primers, Probe or 20× ROX Reference Dye to 2 × QuickEasy™ Real PCR Mix-Taqman, and dilute it to 1× with DNase-Free ddH_2O (see Table 1 for PCR system preparation).

Note: This operation should be performed on an ice bath, as prolonged room temperature storage will degrade product performance.

Table 1: PCR reaction system preparation

PCR system adding content	Volume	Concentration
2× QuickEasy™ Real PCR Mix-Taqman	10 μL	1×
Forward Primer(10 μM)	0.8 μL	50-900 nM [1*]
Reverse Primer(10 μM)	0.8 μL	50-900 nM [1*]
Probe(4 μM)	1 μL	200 nM

Template	X μL	2*
20× ROX Reference Dye	-	3*
DNase-Free ddH_2O	(7.4-X) μL	
Total Volume	20 μL	

1*： Usually a final primer concentration of 400 nM gives good results. When the reaction performance is poor, the primer concentration can be adjusted in the range of 50-900 nM.

2*: The amount of template added varies depending on the copy number of the target gene present in the template solution, and a gradient dilution is performed to explore the appropriate amount of template added. The amount of template DNA added is preferably below 100 ng. When using RT-PCR reaction cDNA (RT reaction solution) as the template, the amount added should not exceed 10% of the total volume of the PCR reaction solution.

Note: The qPCR system can be adjusted according to the experimental needs and PCR model. The final concentration of most specific primers, we recommend 400 nM, and the final concentration of Probe we recommend 200 nM. Please adjust the dosage of specific primer and Probe according to the prepared concentration according to our recommended final concentration. For qPCR in a 50 μL system, please adjust the amount of reagents according to the proportion of the 20 μL system.

3*: Select the appropriate final concentration of ROX Reference Dye according to different quantitative PCR instruments. The optimum concentration of ROX Reference Dye for common quantitative PCR instruments is shown in the following table:

Real Time-qPCR	ROX Reference Dye Concentration
ABI PRISM7000/7300/7700/ 7900HT/Step One etc.	1× (20 μL system，add 1 μL 20×ROX Reference Dye)
ABI 7500/7500 Fast and Stratagene Mx3000P/Mx3005P/Mx4000 etc.	0.5× (20 μL system，add 0.5 μL 20×ROX Reference Dye)

B: Real Time PCR reaction

Prepare the PCR system according to step A, mix well, and carry out the PCR reaction according to the optimized PCR conditions (annealing temperature, etc.) (reaction conditions are shown in Table 2 below).

Table 2: Real Time PCR-Taqman reaction conditions

Step	Temperature	Time	Cycles	Content
1	94℃	3min	1	Predenaturation
2(2 steps)	94℃	5-10sec	40	In-loop template denaturation
	60-65℃	20-30sec		Annealing/Extension

Note: In order to obtain the best PCR effect, gradient PCR can be used to optimize the reaction conditions for different templates and different primers. PCR reaction conditions vary depending on the quantitative PCR instrument, template, primers, etc. In the specific operation, it is necessary to design the optimal reaction conditions, including annealing temperature, reaction time, etc., according to the specific conditions of quantitative PCR instrument, template type, target fragment size, base sequence of amplified fragment, and GC content and length of primers.

Issues Analysis Guide

The following is an analysis of the problems that may be encountered in the experiments of the fluorescence quantitative PCR 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: 028-83360257 or E-mali: Tech@foregene.com.

No amplified signal

1. The hot-start Taq DNA Polymerase in the kit loses its activity due to improper storage or expiration of the kit.

Recommendation: Confirm the storage conditions of the kit; re-add an appropriate amount of hot-start Taq DNA Polymerase to the PCR system or purchase a new Real Time PCR Kit for related experiments.

2. There are a lot of inhibitors of Taq DNA Polymerase in the DNA template.

Recommendation: Repurify the template or reduce the amount of template used.

3. Mg^{2+} concentration is not suitable.

Recommendation: We provide 2× QuickEasy™ Real Time PCR Mix with a Mg^{2+} concentration of 3.5 mM. However, some special primers and templates may require a higher Mg^{2+} concentration, so $MgCl_2$ can be directly added to optimize the Mg^{2+} concentration. It is recommended to increase 0.5mM Mg^{2+} each time for optimization.

4. The PCR amplification conditions are not suitable, and the primer sequence or concentration is inappropriate.

Suggestion: confirm the correctness of the primer sequences and that the primers are not degraded; if the amplification signal is not good, try to reduce the annealing temperature and adjust the primer concentration appropriately.

5. Template usage problem, too little or too much.

Recommendation: The template linearization gradient dilution can be performed, and the template concentration with the best PCR effect can be selected for the Real Time PCR experiment.

Excessive fluorescence value of NTC

1. Reagent contamination caused during operation.

Recommendation: Replace with new reagents for Real Time PCR experiments.

2. Contamination occurred during the preparation of the PCR reaction system.

Recommendation: Take necessary protective measures during operation, such as: wearing latex gloves, using a pipette tip with a filter element, etc.

3. Primers are degraded, which can lead to non-specific amplification.

Suggestion: SDS-PAGE electrophoresis can be used to detect whether the primers are degraded, and new primers can be used for Real Time PCR experiments.

Primer-dimers or non-specific amplification

1. Mg^{2+} concentration is not suitable.

Recommendation: We provide 2× QuickEasy™ Real Time PCR Mix with a Mg^{2+} concentration of 3.5 mM. However, some special primers and templates may require a higher Mg^{2+} concentration, so $MgCl_2$ can be directly added to optimize the Mg^{2+} concentration. It is recommended to increase 0.5mM Mg^{2+} each time for optimization.

2. PCR annealing temperature is too low.

Recommendation: Optimize the PCR annealing temperature by increasing 1°C or 2°C each time.

3. The PCR product is too long.

Recommendation: The length of the Real Time PCR product should preferably be between 100-150bp, and should not exceed 500bp.

4. The primers are degraded, and the degradation of the primers will lead to the appearance of very specific amplification.

Recommendation: SDS-PAGE electrophoresis can be used to detect whether the primers are degraded, and new primers can be used for Real Time PCR experiments.

5. The PCR system is improper, or the system is too small.

Recommendation: PCR reaction system is too small will lead to lower detection accuracy. It is

best to repeat the Real Time PCR experiment using the reaction system recommended by the quantitative PCR machine.

Poor repeatability of quantitative values

1. Instrument failure.

Recommendation: There may be errors between each PCR well of the instrument, resulting in poor reproducibility during temperature management or detection. Please check according to the instruction manual of the corresponding instrument.

2. The sample purity is not good.

Recommendation: Impure samples will lead to poor reproducibility of experiments, including the purity of templates and primers. Repurification of the template is best done, and primers are best purified using SDS-PAGE.

3. PCR system preparation and storage time is too long.

Recommendation: The Real Time PCR system should be used for PCR experiments immediately after preparation, and should not be put on hold for too long.

4. The PCR amplification conditions are not suitable, and the primer sequence or concentration is inappropriate.

Recommendation: confirm the correctness of the primer sequences and that the primers are not degraded; if the amplification signal is not good, try to reduce the annealing temperature and adjust the primer concentration appropriately.

5. The PCR system is improper, or the system is too small.

Recommendation: PCR reaction system is too small will lead to lower detection accuracy. It is best to repeat the Real Time PCR experiment using the reaction system recommended by the quantitative PCR machine.

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