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One-step Probe RT-qPCR Kit

Cat. No.: SLPCR228

Description

The One-step Probe RT-qPCR Kit is designed for quantitative PCR detection using RNA as a template, such as viral RNA. Using gene-specific primers (GSP), reverse transcription and qPCR reactions are performed in a single tube, requiring no additional tube opening/pipetting, significantly increasing detection throughput and reducing the risk of contamination. This Kit introduced dUTP/UDG system. Heat-labile UDG can rapidly degrade the pollutants containing U at room temperature. When reverse transcription is performed at 55°C, heat-labile UDG will be inactivated rapidly without affecting the efficiency and sensitivity of RT-qPCR. Integrating the superior performance of Reverse Transcriptase and hot-start Taq DNA Polymerase, with the optimized buffer system, the detection sensitivity of One-step Probe RT-qPCR Kit can reach 0.1 pg total RNA or <10 copies of RNA template. The kit is available as a convenient Master Mix. 5 × One-step U⁺ Mix contains an optimized buffer system and dNTP/dUTP Mix, which is suitable for high-specificity, low template concentration and multiple detection systems with fluorescent labeled probes such as TaqMan®. This product is perfectly compatible with common quantitative PCR instruments, such as ABI, Roche, Bio-Rad, etc.

Components

Component	SLPCR228-1 200 rxn, 20 μl/rxn	SLPCR228-2 1000 rxn, 20 μl/rxn
5 × One-step U⁺ Mix[a]	800 μl	1 ml × 4
One-step U⁺ Enzyme Mix V3[b]	200 μl	1 ml
RNase-free ddH_2O	1 ml	15 ml

a. Contains dNTP/dUTP Mix, Mg^{2+}.

b. Contains Reverse Transcriptase, RNase inhibitor, Heat-labile UDG and hot-start Taq DNA Polymerase.

Storage

This reagent should be kept at -20°C.

Applications

- Probe gene expression analysis
- Probe Low-copy gene detection
- Probe microarray validation
- Probe gene knockdown validation

Features

- One-step RT-qPCR
- This kit is suitable for fluorescence quantification by probe method
- This kit is compatible with many real-time systems
- Hot-start technology brings high specificity and reproducible amplification
- dUTP/UDG system, effectively prevent PCR product contamination

Notes

1. One-Step U⁺ Enzyme Mix V3 contains high concentration of glycerol. Before use, please centrifuge briefly, collect to the bottom of the reaction tube, and gently suck with a pipetting gun.
2. For preparation of reaction solution, please use RNase-free pipette tips, EP tubes, etc., to avoid contamination as far as possible.

Protocol

1. Preparation of reaction solution (with ABI StepOnePlus as the test model)

Add the following reagents to the proper thermal cycler reaction tube or plate on ice:

Component	Volume	Final concentration
5 × One-step U⁺ Mix	4 μl	1 ×
One-step U⁺ Enzyme Mix V3	1 μl	_
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
Probe (10 μM)	0.2 μl	0.1 μM
Template RNA	1 pg-1 μg	1 pg-1 μg/20 μl
RNase-free ddH_2O	to 20 μl	_

The amount of each component in the reaction system can be adjusted according to the following principles:

- The optimal range for primers is 0.1~1.0 μM. In general, the primers with a final concentration of 0.2 μM work well.
- The optimal range for probes is 50-250 nM.
- QPCR is highly sensitive, and the accuracy of the amount of template added to the reaction system will have a great impact on the final quantitative results. It is recommended to add the template to the reaction system after dilution (such as dilution to 2-5 μl/ sample), which can effectively improve the repeatability of the experiment.
- The length of the amplification product should be in the range of 80-200 bp.

2. Preform One-step RT-qPCR using the following thermal cycling condition

Set the thermal cycling conditions using default RT-qPCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters of the specific primers.

Standard RT-qPCR mode (maximum amplification sensitivity) :

Reverse transcription	55°C [a]	15 min	/
Initial denaturation	95°C	30 sec	/

Circular reaction	95°C	10 sec	45 Cycles
	60°C	30 sec [b]	

Fast RT-qPCR mode (for most applications):

Reverse transcription	50°C [a]	2-5 min	/
Initial denaturation	95°C	2-15 sec	/
Circular reaction	95°C	1-10 sec	40-45 Cycles
	60°C	10-20 sec [c]	

a. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55ºC is conducive to improving amplification efficiency and sensitivity.

b. The extension time should be adjusted according to the minimum time limit of data collection required by the Real Time PCR instrument you use: at least 30 seconds with ABI 7700 and 7900HT; at least 31 seconds when using ABI 7000 and 7300; use ABI 7500 for at least 34 seconds.

c. Whether the Real Time PCR instrument actually used supports rapid amplification cycle or not, please conduct preliminary experiment to confirm the initial attempt.

3. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucl- eases and ribonucleases is confirmed by appropriate quality tests.

Functional test: the total RNA of HeLa cells was used as the template to amplify 2 different abundance genes with 4 concentration gradients in 1 pg-1μg starting volume. The amplification efficiency was between 0.9 and 1.1, and the CT value of B2M gene was within 35 when the template amount was 1pg.

Product Use Limitations

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.