SuperFast Probe One Step

RT-qPCR U+ Kit

Catalog number: S666097 (200 rxns)

Storage condition: -30℃ ~-15℃ for storage, ≤0℃ for transportation.

Products content

Component	200 rxns
5×SuperFast One Step RT-qPCR U+ Buffer	1 mL
SuperFast One Step U+ Enzyme	200 μL
RNase-Free Water	2 x 1.5 mL

Products Introduction

The SuperFast Probe One Step RT-qPCR U+ Kit is designed for quantitative PCR assays using RNA as a template (e.g., RNA viruses). Using gene-specific primers (GSP), reverse transcription and qPCR reactions are completed in a single tube, eliminating the need for additional tube-opening/pipetting operations, greatly increasing throughput and reducing the risk of contamination. The dUTP/UNG anti-contamination system is introduced in this kit. The heat-sensitive UNG rapidly degrades U-containing contaminants at room temperature; it is rapidly inactivated by reverse transcription at 55°C, without affecting the efficiency and sensitivity of qRT-PCR. Combined with optimized buffer systems and antibody-modified Taq enzymes and mutated M-MLV, the SuperFast Probe One Step RT-qPCR U+ Kit provides sensitivity up to 0.1 pg of total RNA or <10 copies of RNA template and enhanced thermal stability. 5× SuperFast One Step RT-qPCR U+ Buffer contains the following components The 5× SuperFast One Step RT-qPCR U+ Buffer contains an optimized buffer system and dNTP/dUTP Mix, which is particularly suitable for high specificity, low template concentration and multiplexed rapid detection of fluorescently labeled probes such as TaqMan.

caveat

Before use, please mix the product gently by turning it up and down after it is completely melted to avoid foaming, and use it after brief centrifugation. Avoid repeated freezing and thawing of the product.ROX dye is used to correct the fluorescence signal error between the quantitative PCR wells, this product does not contain ROX dye, if you need to match the ROX dye with the instrument you are using, please contact your local business or call CombiSense customer service at 4006-222-360.

PCR reaction system

reagents	50 μL system	25 μL system	final concentration
5×SuperFast One Step RT-qPCR U+ Buffer	10 μL	5 μL	
SuperFast One Step U+ Enzyme	2 μL	1 μL	1×
Forward Primer, 10 μM	1 μL	0.5 μL	0.2 μM1)
Reverse Primer, 10 μM	1 μL	0.5 μL	0.2 μM
Probe2	0.5 μL	0.25 μL	0.1 μM
Template RNA3)	X μL	X μL	
RNase-Free Water	Up to 50 μL	Up to 25 μL	

Attention:

(1) Usually, the final primer concentration of 0.2 μM can get better results, and 0.1-1.0 μM can be used as a reference for setting the range. If the amplification efficiency is not high, the concentration of primer can be increased; if non-specific reaction occurs, the concentration of primer can be decreased to optimize the reaction system.

(2) The final concentration of the probe used is related to the fluorescence quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance, please refer to the instrument manual or the specific requirements for the use of each fluorescent probe to adjust the concentration.

3) Because templates from different species contain different numbers of copies of the target gene, the template can be diluted in a gradient to determine the optimal amount of template to use

PCR reaction conditions

move	temp	timing	circulate
reverse transcription	55°C	1 min	1
premutability	95°C	10s1)	1
denaturation	95°C	1 s	40-45
Annealing/Extension	55-60°C2)	10-15s3)	40-45

Attention:

(1) The enzyme used in this product is activated under the condition of pre-denaturation at 95℃ for 30s. Under this condition, most of the templates can be well unchained. For templates with high GC content and complex secondary structure, the pre-denaturation time can be extended to 1min, so as to make the starting template fully unchained, and if the high temperature treatment time is too long, it will affect the activity of the enzyme; for simple templates, pre-denaturation time of 1-10s can also be used, and the optimal pre-denaturation time can be determined according to the template situation.

(2) It is recommended to use two-step PCR reaction program, the annealing temperature should be 55-60℃ as the reference range, and the annealing temperature can be increased when non-specific reaction occurs. If you can't get good results due to the use of primers with low Tm values or long amplification products, you can try three-step PCR amplification.

3) Whether the actual Real Time PCR instrument used supports rapid amplification cycles, please perform a pre-experiment to verify this for the first attempt.