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Fast Probe One Stept RT-qPCR, UNG Kit

Product Number:PCK27

Shipping and Storage

-30°C~ -15°C, transport ≤0°C.

Component

Component	PCK27 200rxns
5×Fast Probe One Stept RT-qPCR, UNG KitBuffer	1mL
Fast Probe One Stept RT-qPCR, UNG Kit Enzyme	200μL
RNase-Free Water	2×1.5mL

Description

Fast Probe One Stept RT-qPCR, UNG Kit is designed specifically for quantitative PCR detection using RNA as a template, such as RNA viruses. Using gene-specific primers (GSPS), reverse transcription and qPCR reactions are performed in one tube without the need for additional tube opening/pipetting operations, greatly increasing assay throughput and reducing the risk of contamination. The dUTP/UNG anti-contamination system is introduced in this kit. Heat sensitive UNG can degrade U-containing pollutants rapidly at room temperature. Rapid inactivation of UNG by reverse transcription at 55°C did not affect the efficiency and sensitivity of qRT-PCR. Combined with optimized buffer system, antibody modified Taq enzyme and mutated M-MLV, the detection sensitivity of Fast Probe One Stept RT-qPCR, UNG Kit can reach 0.1pg total RNA or <10 copies of RNA template and has higher thermal stability. 5×Fast Probe One Stept RT-qPCR, UNG Kit Buffer contains optimized buffer system and dNTP/dUTP Mix, which is especially suitable for high specificity, low template concentration and multiple fast detection of fluorescent-labeled probes such as TaqMan.

Note

Before use, please gently mix the product upside down after it is completely melted, avoid foaming as far as possible, and use after a short centrifugation. Avoid freezing and thawing this product repeatedly. ROX dye is used to correct fluorescence signal errors generated between quantitative PCR holes. This product does not contain ROX dye.

PCR Reaction System

Reagent	50μL	25μL	Final Conc.
5×Fast Probe One Stept RT-qPCR, UNG KitBuffer	10 μL	5 μL	1×
Fast Probe One Stept RT-qPCR, UNG Kit Enzyme	2 μL	1 μL	
Forward Primer 10 μM	1 μL	0.5 μL	0.2 μM[1)]
Reverse Primer 10 μM	1 μL	0.5 μL	0.2 μM
Probe[2)]	0.5 μL	0.25 μL	0.1 μM
Template RNA[3)]	X μL	X μL	
RNase-Free Water	Up to 50 μL	Up to 25 μL	

Note:1) Generally, the final primer concentration of 0.2μM can get better results, and 0.1-1.0μM can be used as a reference for the set range. When the amplification efficiency is not high, the concentration of primers can be increased. When non-specific reaction occurs, the concentration of primers can be reduced 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 labeled substance. Please adjust the concentration according to the instructions of the instrument or the specific requirements for the use of each fluorescent probe.

3) Due to the different number of target gene copies contained in the templates of different species, gradient dilution of the templates can be carried out to determine the optimal use of templates.

PCR reaction condition

Step	Temperature	Time	cycles
Reverse transcription	55°C	1 min	1
Pre denaturation	95°C	10s1)	1
Denaturation	95°C	1 s	40-45
Annealing/Extension	55-60°C2)	10-15s3)	

Note:1) The enzyme used in this product can be activated under the condition of pre-denaturation at 95°C for 30s. Under these conditions, most templates can be unchained well. For templates with high GC content and complex secondary structure, the pre-denaturation time can be extended to 1min to make the initial template fully unchain. If the processing time is too long at high temperature, the enzyme activity will be affected. For simple templates, 1-10s can also be used to determine the best pre-denaturation time according to the template conditions.

2) It is recommended to adopt the two-step PCR reaction procedure, and the annealing temperature should be 55-60°C as the reference of the setting range. When the non-specific reaction occurs, the annealing temperature can be increased. If the primers with low Tm value or the amplified products are too long and the experimental results are not good, the three-step PCR amplification can be attempted.

3) Please verify whether the Real Time PCR instrument actually used supports the rapid amplification cycle before the first experiment.