FastDirect Probe RT-qPCR Premix plus-UNG

Cat.No.:HYA433

Product Description

FastDirect Probe RT-qPCR Premix plus-UNG is a specialized reagent designed for direct fluorescent quantitative (TaqMan probe-based) RNA amplification detection of target genes, eliminating the need for nucleic acid extraction and purification from samples. It contains a genetically engineered and selected rapid-amplification reverse transcriptase and DNA polymerase, enabling PCR reactions to be completed within 20–40 minutes. This product exhibits strong inhibitor tolerance, allowing direct RNA amplification detection from samples such as anticoagulated whole blood, plasma, serum, throat swabs, and feces without prior nucleic acid extraction and purification. Formulated with an optimized qPCR-specific Buffer and a UNG/dUTP anti-contamination system, this reagent effectively prevents false-positive amplification caused by PCR product carryover and aerosol contamination.

Components

1. 10×Fast Direct RTase/UNG Mix
2. 2×Fast Direct RT Premix Buffer (dUTP)

Storage

Store at -20℃ for long-term storage. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Instructions

1. Reaction system

Components	25 uL Volume	50 uL Volume	Final Concentration
2×Fast Direct RT Premix Buffer (dUTP)	12.5 μL	25 μL	1×
10×Fast Direct RTase/UNG Mix	2.5 μL	5 μL	1×
25×Primer-Probe Mix*	1 uL	2 uL	1×
Template RNA**	-	-	-
DEPC H_2O	Up to 25 μL	Up to 50 μL	-

*When using conventional PCR protocols for amplification, a final primer concentration of 0.2 μM typically yields satisfactory results; if the reaction performance is unsatisfactory, the primer

concentration can be adjusted within the range of 0.2–1 μM. Generally, the probe concentration is optimized within the range of 0.1–0.3 μM. Concentration gradient experiments can be performed to identify the optimal combination of primers and probes. When using rapid PCR protocols for amplification, appropriately increasing the concentrations of primers and probes may yield better amplification results, and the primer-probe ratio should undergo ratio optimization.

**The types, contents of inhibitors, and copy numbers of target genes vary among different types of biological samples; variations also exist in the impact of different types of sample preservation solutions (e.g., swab preservation solutions) on amplification performance. Therefore, the optimal sample volume in the reaction system should be determined based on actual conditions such as sample type and detection sensitivity requirements. If necessary, the sample can be diluted with nuclease-free water or TE Buffer before adding it to the reaction system. Recommended sample volumes are as follows:

Type of Sample	Added Volume for 50 μL Reaction System	Maximum Added Sample Ratio
Whole blood	2.5 μL	5%
Throat Swab	10 uL	20%

2. Reaction program

Regular PCR program			
Step	**Temperature**	**Time**	**Cycles**
Reverse transcription	50℃	10-20 min	1
Pre-denaturation	95℃	1-5 min	1
Denaturation	95 ℃	10-20 s	40-50
Annealing and Extension	56~64℃	20-60 s	

Rapid PCR program			
Step	**Temperature**	**Time**	**Cycles**
Reverse transcription	50℃	5 min	1
Pre-denaturation	95℃	30 s	1
Denaturation	95 ℃	1-3 s	40-45
Annealing and Extension	56~64℃	3-20 s	

Quality Control

1. Functional Testing: qRT-PCR sensitivity, specificity, and repeatability.
2. Free of extraneous nuclease activity, as well as extraneous endonuclease and exonuclease contamination.

Technical Information

1. The amplification rate of the DNA polymerase contained in this product is no less than 1 kb/10 s. Significant variations exist in temperature ramping rate, temperature control mode, and heat transfer efficiency among different PCR instruments. It is recommended to optimize the optimal reaction parameters in conjunction with the specific rapid PCR instrument used.
2. If issues such as low fluorescence values or significant amplification inhibition occur, it is recommended to reduce the sample volume or dilute the sample before adding it to the reaction system.
3. The collection of samples such as blood, throat swabs, and feces mentioned above shall be performed in accordance with clinical standard operating procedures (SOPs); to avoid nucleic acid degradation, freshly collected samples are recommended.
4. The reverse transcription time should be optimized based on the length of the target fragments; appropriately prolonged reverse transcription time is recommended for longer fragments.
5. The annealing-extension temperature should be optimized according to the Tm values of the primers and probes, as well as the actual reaction conditions.
6. For primers with low annealing temperatures or amplification of long fragments exceeding 200 bp, the three-step PCR protocol is recommended.
7. Different target genes vary in their dUTP utilization efficiency and sensitivity to UNG enzyme. Therefore, if the use of the UNG system leads to decreased detection sensitivity, the reaction system should be adjusted and optimized accordingly. For technical support, please contact our company.
8. Use dedicated areas and pipettes for pre- and post-amplification steps. Operate with gloves and replace them frequently. Do not open the reaction tubes after PCR completion to minimize contamination of samples by PCR amplicons.
9. For Research Use Only !