Cat.:MD2084-4

AcuGenix™ Fast Direct One-Step RT-qPCR Master Mix with UNG-V4 V02

Product Description

AcuGenix™ Fast Direct One-Step RT-qPCR Master Mix with UNG-V4 is designed for direct target gene RNA amplification and detection by quantitative fluorescent PCR (TaqMan probe method), eliminating the need for nucleic acid extraction and purification. It contains a genetically engineered and screened reverse transcriptase and DNA polymerase that enable rapid amplification, completing the PCR reaction in just 20–40 minutes. The reagent exhibits high tolerance to inhibitors, allowing direct RNA amplification and detection from samples such as throat swabs, anticoagulated whole blood, plasma, and serum without nucleic acid extraction and purification. The kit employs an optimized qPCR-specific buffer and a UNG/dUTP contamination prevention system, effectively preventing false-positive amplifications caused by PCR product carryover and aerosol contamination.

Components

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

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Prepare Reaction Mix

Composition	Volume per Reaction	Volume per Reaction	Final Concentration
2×Fast Direct RT Premix Buffer Ⅳ (dUTP)	12.5 μL	25 μL	1×
10×Fast Direct RTase/UNG Mix Ⅳ	2.5 μL	5 μL	1×
25×Primer-Probe Mix [1, 2]	1 μL	2 μL	1×
Sample [3]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. The final concentration of primers is usually 0.2 μM for better results. when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. The probe concentration is usually optimized in the range of 0.1-0.3 μM. Concentration gradient experiments can be performed to find the best combination of primers and probes.
2. When using a fast PCR program, appropriately increasing the primer and probe concentrations may achieve better amplification results. The ratio of primers to probe should be optimized accordingly.
3. Different types of biological samples vary in the type and content of inhibitors as well as the copy number of target genes. Moreover, different sample preservation solutions (e.g., swab preservation solutions) have different effects on the amplification reaction. Therefore, the optimal sample loading volume in the reaction system should be determined based on the sample type and the required detection sensitivity. If necessary, samples may be diluted with nuclease-free water or TE Buffer before loading. Recommended loading volumes are as follows:

Sample Type	Volume per 50 μL Reaction	Maximum Sample Loading Ratio
Anticoagulated whole blood	2.5 μL	5%
Plasma	15 μL	30%
Serum	10 μL	20%
Throat swab	10 μL	20%
20% fecal suspension	0.5 μL	1%

Reaction Program

Standard PCR Program			
Procedure	Temp.	Time	Cycles
Reverse Transcription	50℃	5-10 min	1
Hot Start	95℃	30s-5 min	1
Denaturation	95℃	10-20 s	40-50
Annealing and Extension	56-64℃	20-60 s	

Fast PCR Program			
Procedure	Temp.	Time	Cycles
Reverse Transcription	50℃	2 min	1
Hot Start	95℃	30 s	1
Denaturation	95℃	1-3 s	40-45
Annealing and Extension	56-64℃	3-20 s	

*The temperature-sensitive TS-UNG enzyme contained in this product is active at room temperature and is inactivated during the reverse transcription process.

Quality Control

1. Functional testing: sensitivity, specificity, reproducibility of qPCR.
2. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

Notes

1. The rapid DNA polymerase contained in this product has an amplification rate of no less than 1 kb per 10 seconds. Different PCR instruments vary significantly in ramping rate, temperature control mode, and heat transfer efficiency. It is recommended to optimize the reaction conditions according to the specific fast PCR instrument used.
2. If issues such as low fluorescence signal or significant amplification inhibition occur, it is recommended to reduce the sample loading volume or dilute the sample before adding it to the reaction.
3. The collection of the above-mentioned samples, such as blood and throat swabs, should follow standard clinical operating procedures. To avoid nucleic acid degradation, freshly collected samples are recommended.
Reverse transcription time should be optimized based on the length of the target amplicon; longer fragments may require appropriately extended reverse transcription time.
4. The annealing/extension temperature should be optimized according to the Tm values of the primers and probe and the actual reaction conditions.
5. For primers with low annealing temperatures or for amplification of long fragments exceeding 200 bp, a three-step PCR protocol is recommended.
6. Different target genes vary in their efficiency of dUTP utilization and their sensitivity to UNG enzyme. Therefore, if the use of the UNG system results in reduced detection sensitivity, the reaction system should be adjusted and optimized. For technical support, please contact our company.
7. Use dedicated areas and pipettes before and after amplification; wear gloves and change them frequently. Do not open the reaction tubes after PCR amplification to minimize contamination of samples by PCR products.