Cat.:M5144-NA

AcuGenix™ FastAmpli RT-qPCR Master Mix with UI (Low DNA)

V01

Product Description

AcuGenix™ FastAmpli RT-qPCR Master Mix with UNG (Low DNA) is suitable for fluorescence quantitative (TaqMan probe method) RNA amplification detection. It contains rapidly amplified reverse transcriptase and DNA polymerase screened by genetic modification, and can complete the PCR reaction within 20-40 min. This product has strong inhibitor tolerance, higher reverse transcription and PCR amplification efficiency, and is suitable for high-sensitivity amplification of low-concentration RNA templates. Good standard curves can be obtained in a wide quantitative region to accurately quantify. This product consists of an anti-inhibitory amplification enzyme, UNG enzyme, and an optimized buffer system containing dUTP to prevent false positive reactions caused by residual PCR products or aerosol contamination. This product is compatible with fluorescence quantitative PCR instruments from most manufacturers, such as Applied Biosystems, Eppendorf, Bio-Rad and Roche. Through specialized treatment, this product is devoid of DNA residues and is appropriate for the development of biopharmaceutical-related detection reagents.

Components

1. 10×FastAmpli RTase/UNG Mix
2. 5×FastAmpli RT Premix Buffer (dUTP) (Mg^{2+} free) (DG)
3. 25×$MgCl_2$

Storage

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

Prepare Reaction Mix

Components	Volume per Reaction	Volume per Reaction	Final Concentration
5×FastAmpli RT Premix Buffer (dUTP) (Mg^{2+} free) (DG)	5 μL	10 μL	1×
10×FastAmpli RTase/UNG Mix	2.5 μL	5 μL	1×
25×$MgCl_2$	1 μL	2 μL	1×
25×Primer-Probe Mix [1,2]	1 μL	2 μL	1×
Template RNA [3]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. When using ordinary PCR program amplification, usually primer final concentration of 0.2μM can get better results ; when the reaction performance is poor, the primer concentration can be adjusted in the range of 0.2-1 μM. Generally, the probe concentration is optimized in the range of 0.1-0.3 μM. Concentration gradient experiments can be carried out to find the best combination of primers and probes.
2. When using rapid PCR program amplification, it is possible to obtain better amplification results by appropriately increasing the concentration of primers and probes, and the ratio of primers and probes should be optimized.
3. The copy number of target genes contained in different types of templates differs; therefore, it may be necessary to perform gradient dilution experiments to determine the optimal amount of template addition.

Reaction Programe

Standard PCR Protocol			
Procedure	Temp.	Time	Cycle
Reverse transcription	50℃	10-20 min	1
Hot-start	95℃	1-5 min	1
Denaturation	95℃	10-20 s	40-50
Annealing /Extension	56-64℃	20-60 s	

FAST PCR Protocol			
Procedure	Temp.	Time	Cycle
Reverse transcription	50℃	5 min	1
Hot-start	95℃	30 s	1
Denaturation	95℃	1-3 s	40-45
Annealing /Extension	56-64℃	3-20 s	

*The temperature-sensitive (TS) UNG enzyme incorporated in this product exhibits activity at ambient temperature and undergoes inactivation during the reverse transcription procedure.

Quality Control

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

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Notes

1. The amplification rate provided by DNA polymerase contained in this product is not less than 1 kb/10 s. Different fast PCR instruments have different temperature control modes, rates, and thermal conductivity efficiencies. It is recommended that specific optimization for each instrument's most suitable reaction conditions be carried out along with their respective fast PCR instruments.
2. The reverse transcription time should be optimized according to the length of the fragment to be amplified, and longer fragments may require appropriately extended reverse transcription time.
3. The annealing and extension temperature should be optimized based on the Tm value of the primers and probes, as well as the actual reaction conditions.
4. For primers with low annealing temperatures or amplicon fragments longer than 200 bp, it is recommended to use a three-step method for amplification.
5.The efficiency of dUTP utilization and sensitivity to UNG enzyme varies for different genes to be amplified. If the detection sensitivity is reduced due to the use of the UNG system, adjust and optimize the reaction system accordingly. If technical support is required, please contact our company.
6. Use dedicated areas and pipettes before and after amplification, wear gloves during operation, and regularly replace them. Do not open reaction tubes after PCR completion to minimize sample contamination from PCR products.