Cat.:FMD5084-4

AcuGenix™ Fast Direct Lyo-Ready RT-qPCR Master Mix with UNG-V4 V01

Product Description

AcuGenix™ Fast Direct Lyo-Ready 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. 12.5×Fast Direct Part Ampli/UNG Mix Ⅳ (with dNTPs) (DG)
2. 50×Fast Direct Part RTase Ⅳ (DG)
3. 5×Fast Direct RT Buffer Ⅳ (DG)
4. 4×AcuGenix™ Lyophilization Protectant-9 (optional)

Storage

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

Prepare Reaction Mix

Composition	Volume per Reaction	Volume per Reaction	Final Concentration
5×Fast Direct RT Buffer Ⅳ (DG)	5 μL	10 μL	1×
12.5×Fast Direct Part Ampli/UNG Mix Ⅳ (with dNTPs) (DG)	2 μL	4 μL	1×
50×Fast Direct Part RTase Ⅳ (DG)	0.5 μL	1 μL	1×
4×AcuGenix™ Lyophilization Protectant-9 [1]	6.25 μL	12.5 μL	1×
25×Primer-Probe Mix [2,3]	1 μL	2 μL	1×
Template RNA [4]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. This system can be lyophilized, when there is no need to do so, 4×AcuGenix™ Lyophilization Protectant-9 can be selectively added during use. If there is a need to produce lyophilized products, during liquid testing stage verification of product performance must include 4×AcuGenix™ Lyophilization Protectant-9 to ensure consistency with the composition and effects after lyophilization.
2. 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.
3. 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.
4. Biological sample types vary in their inhibitor composition, inhibitor concentration, and target gene copy number. The effect of sample storage media (e.g., swab transport media) on amplification also differs. The optimal sample input volume should be determined according to sample type and required detection sensitivity. When necessary, samples may be pre-diluted with nuclease-free water or TE buffer. Recommended volumes for a 50 μL reaction: anticoagulated whole blood 2.5 μL (5%), plasma 10 μL (30%), serum 10 μL (30%), nasopharyngeal swab 10 μL (20%), saliva 10 μL (20%).

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	

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

Prepare Reaction Mix for lyophilization

Composition	Volume per Reaction (25 μL)
12.5×Fast Direct Part Ampli/UNG Mix Ⅳ (with dNTPs) (DG)	2 μL
50×Fast Direct Part RTase Ⅳ (DG)	0.5 μL
4×AcuGenix™ Lyophilization Protectant-9	6.25 μL
25×Primer-Probe Mix	1 μL
ddH_2O	To 18-20 μL

1.This product is not recommended for lyophilization of all components; Component 5×Fast Direct RT Buffer Ⅳ (DG) is used as a reconstitution solution.

2.For other lyophilization system formulations, please inquire separately.

Lyophilization conditions

Procedure	Temperature	Time	status	Pressure
Pre-freezing	4℃	30 min	Hold	1 atm
	-50℃	60 min	Cooling	
	-50℃	180 min	Hold	
Primary drying	-30℃	60 min	Heating	Ultimate vacuum
	-30℃	720 min	Hold	
Secondary drying	25℃	60 min	Heating	Ultimate vacuum
	25℃	300 min	Hold	

1. This freeze-drying process is an in-situ freeze-drying process for a 25 μL system. If need to perform freeze-drying of freeze-dried beads or other in-situ systems, please inquire separately.

2. The above freeze-drying process is for reference only. Different product types and the use of different freeze dryers may result in different parameters, so adjustments can be made based on actual usage.

Quality Control

1. Functional testing: qPCR sensitivity, specificity, repeatability.

2. No exogenous nuclease activity, no exogenous endonuclease/exonuclease contaminatio.

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. 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, should be performed in accordance with standard clinical operating procedures. To avoid nucleic acid degradation, freshly collected samples are recommended.

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 enzy、me 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.