Cat.:FM2181-4-NA

AcuGenix™ FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG (Low DNA)-V4 V01

Product Description

AcuGenix™ FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG (Low DNA)-V4 is a specialized reagent for Real Time PCR qualitative and quantitative reactions using the probe method, developed specifically for lyophilization processes. It contains genetically modified DNA polymerase that can rapidly amplify DNA and complete the entire PCR reaction within 30 minutes. The buffer system has been optimized for multiple amplifications. This reagent contains an enzyme mixture specifically designed for lyophilized systems, which has been repeatedly optimized and does not require additional lyoprotectants. This reagent contains an enzyme mixture specifically designed for lyophilized systems, which has been repeatedly optimized and does not require additional lyoprotectants. 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 reagent is compatible with most real-time PCR instruments from major manufacturers, including 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

AcuGenix™ FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG (Low DNA)-V4

*This reagent already contains contains rapid Taq DNA polymerase, PCR Buffer, UNG, dNTPs (with dUTP instead of dTTP), $MgCl_2$, stabilizer and lyoprotectants.

Storage

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

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
AcuGenix™ FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG (Low DNA)-V4[1]	12.5 μL	25 μL	1×
25×Primer-Probe Mix [2,3]	1 μL	2 μL	1×
Template DNA [4]	—	—	—
ddH_2O	To 25 μL	To 50 μL	—

1. This system is a lyophilizable system that already contains lyoprotectant ingredients, if there are freeze-dried products required, simply add primers/probes to achieve freeze drying.
2. When using conventional PCR programs for amplification, the final concentration of primers should typically be 0.2 μM to obtain better results. The primer concentration can be adjusted within the range of 0.2-1 μM if reaction performance is poor. Optimize the probe concentration within a range of 0.1 to 0.3 μM.Conduct experiments with gradient concentrations to find the best combination of primers and probes.
3. When using fast PCR programs for amplification, increasing the concentration of primers/probes may result in better amplification results. The ratio between primers and probes should be optimized.
4. The copy number of target genes varies in different types of templates, when necessary, dilute gradients to determine the optimal amount of template addition.

Reaction Programe

Standard PCR Protocol			
Procedure	Temp	Time	Cycle
Digestion	50°C	2 min	1
Hot Start	95°C	1-5 min	1
Denaturation	95°C	10-20 s	40-50
Annealing and Extension	56-64°C	20-60 s	

FAST PCR Protocol			
Procedure	Temp	Time	Cycle
Digestion	50°C	2 min	1
Hot Start	95°C	30 s	1
Denaturation	95°C	1-3 s	40-45
Annealing and Extension	56-64°C	3-20 s	

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Prepare Reaction Mix for lyophilization

Components	25 μL System
AcuGenix™ FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG (Low DNA)-V4	12.5 μL
25×Primer-Probe Mix	1 μL
ddH_2O	To 18-20 μL

*For other lyophilization preparation systems, please inquire separately.

Lyophilization Programe

Step	Temperature	Time	Condition	Pressure
Pre-freezing	4℃	30 min	Hold	1 atm
	-50℃	60 min	Cooling	
	-50℃	180 min	Hold	
First Drying	-30℃	60 min	Heating	Ultimate Vacuum
	-30℃	720 min	Hold	
Second 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.
3. Different freeze-drying processes may be suitable for different batch sizes, and sufficient testing and verification must be conducted when used for large-scale production.

Lyophilized Powder Usage Method

1. Briefly centrifuge lyophilized powder.
2. Add nucleic acid template to lyophilized powder and add water up to a total volume of 25 μL.
3. Mix well by centrifugation, and then run on PCR instrument.

* It is recommended that Biori CM-8 or CM-8 Plus Centrifugal mixer be used for standardized mixing operations.

Quality Control

1. Functional testing: qPCR sensitivity, specificity, repeatability.
2. No exogenous nuclease activity, no exogenous endonuclease/exonuclease contamination.

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. This reagent has higher specificity and significantly improves fluorescence quantitative PCR limit detection sensitivity, making it suitable as a high-sensitivity fluorescence quantitative PCR test agent for improving curve uniformity and fluorescent values at extremely low template concentrations.
3. For primers with lower annealing temperatures or fragments over 200 bp, it is suggested that three-step amplification be used.
4. 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.
5. 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.

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