Cat.:FM5144

AcuGenix™ FastAmpli Lyo-Ready RT-qPCR Master Mix with UNG V01

Product Description

AcuGenix™ FastAmpli Lyo-Ready RT-qPCR Master Mix with UNG is a specialized reagent developed specifically for lyophilization processes, designed for fluorescence quantitative (TaqMan probe method) RNA amplification assays. It contains genetically engineered reverse transcriptase and DNA polymerase optimized for rapid amplification, enabling completion of PCR reactions within 20–40 minutes. This reagent exhibits strong tolerance to inhibitors, higher reverse transcription and PCR amplification efficiencies, and is suitable for high-sensitivity amplification of low-concentration RNA templates. It produces reliable standard curves across a wide quantitative range, ensuring accurate quantification. The reagent employs a hybrid enzyme comprising inhibitor-resistant amplification enzymes and UNG enzymes, along with an optimized buffer system containing dUTP, which not only ensures efficient amplification of target genes in inhibitor-containing samples but also effectively prevents false-positive reactions caused by residual PCR products or aerosol contamination. This reagent is compatible with fluorescence quantitative PCR systems from multiple manufacturers, including Applied Biosystems, Eppendorf, Bio-Rad, and Roche. The lyophilized product maintains excellent morphology and stability.

Components

1. 12.5×FastAmpli Part Taq/UNG Mix (with dNTPs) (DG)
2. 50×FastAmpli Part RTase (DG)
3. 5×FastAmpli RT Buffer (DG)
4. 4× lyophilization protective agent (optional)

Storage

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

Reaction Programe

Standard PCR Program				Rapid PCR Procedure			
Step	Temperature	Duration	Cycles	Step	Temperature	Duration	Cycles
UNG treatment	50°C	2 min	1	UNG treatment	50°C	5 min	1
Hot Start	95°C	1-5 min	1	Hot Start	95°C	30s	1
Denaturation	95°C	10-20 s	40-50	Denaturation	95°C	1-3 s	40-50
Annealing and Extension	56-64°C	20-60 s		Annealing and Extension	56-64°C	3-20s	

* The thermosensitive TS-UNG (DG) enzyme contained in this product becomes functional at room temperature and becomes inactive during the reverse transcription process.

Prepare Reaction Mix

Composition	Volume per Reaction	Volume per Reaction	Final Concentration
5×FastAmpli RT Buffer (DG)	5 μL	10 μL	1×
12.5×FastAmpli Part Taq/UNG Mix (with dNTPs) (DG)	2 μL	4 μL	1×
50×FastAmpli Part RTase (DG)	0.5 μL	1 μL	1×
4× lyophilization protective agent (optional)[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 is a lyophilization system. When the customer does not require lyophilization during its use, the 4× lyophilization protective agent may be added selectively. However, if lyophilized products are required, the 4× lyophilization protective agent must be added during product performance validation at the liquid reagent stage to ensure consistency with the components and efficacy of the lyophilized product.
2. When using a standard PCR protocol for amplification, a final primer concentration of 0.2 μM typically yields optimal results; when reaction performance is suboptimal, the primer concentration can be adjusted within the range of 0.2–1 μM. The probe concentration is usually optimized within the range of 0.1–0.3 μM. Concentration gradient experiments can be conducted to identify the optimal combination of primers and probes.
3. When using the rapid PCR protocol for amplification, appropriately increasing the primer-probe concentration may

yield better amplification results; the primer-probe ratio should be optimized accordingly.
4. Different types of templates contain varying copy numbers of the target gene; gradient dilution may be performed when necessary to determine the optimal template addition amount.

Lyophilization System Preparation

Components	25 μL System
5×FastAmpli RT Buffer (DG)	5 μL
12.5×FastAmpli Part Taq/UNG Mix (with dNTPs) (DG)	2 μL
50×FastAmpli Part RTase (DG)	0.5 μL
4× lyophilization protective agent (optional)	6.25 μL
25×Primer-Probe Mix	1 μL
ddH_2O	To 25 μL

*For other lyophilization system preparations, please consult us separately.

Lyophilization Conditions

Step	Temperature	Duration	State	Pressure
Pre-freezing	4℃	30 min	Hold	1 atm
	-50℃	60 min	Heating	
	-50℃	180 min	Hold	
Primary Sublimation	-30℃	60 min	Heating	Ultimate Vacuum
	-30℃	720 min	Hold	
Secondary Sublimation	25℃	60 min	Heating	Ultimate Vacuum
	25℃	300 min	Hold	

Usage Instructions for Lyophilized Powder

1. Perform instantaneous centrifugation of the lyophilized powder;
2. Add the nucleic acid template to the lyophilized powder and supplement with water to a volume of 25 μL;
3. Mix thoroughly, centrifuge, and load onto the instrument.

It is recommended to use the Baotaiyi Company's integrated centrifugal homogenizer CM-8 or CM-8 Plus for standardized centrifugal homogenization operations.

Quality Control

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

Technical Notes

1. Sensi Direct qPCR Master Mix with UNG uses a new type of hot start enzyme that allows for quick activation within 1 to 5 minutes, making it particularly suitable for high-sensitivity multiplex gene detection.
2. If low fluorescence signal or significant amplification inhibition is observed, it is recommended to reduce the sample input volume or pre-dilute the sample before addition. Alternatively, the reaction volume may be increased (60-100 μL) while maintaining the same sample input volume.
3. Collection of blood, saliva, and nasopharyngeal swab samples should be performed in accordance with standard clinical operating procedures. To minimize 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 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.