Cat.:FMD5104-000830

AcuGenix™ SensiDirect Lyo-Ready RT-qPCR Master Mix with UNG-000830

V01

Product Description

AcuGenix™ SensiDirect Lyo-Ready RT-qPCR Master Mix with UNG-000830 is a lyophilization-ready reagent designed for direct RNA amplification and target detection by TaqMan probe-based RT-qPCR without prior nucleic acid extraction or purification. It contains genetically engineered, rapid reverse transcriptase and DNA polymerase and supports completion of RT-qPCR within 20-40 min. The product has strong inhibitor tolerance and can be used directly with samples such as anticoagulated whole blood, saliva, and throat swabs. The formulation combines inhibitor-resistant amplification enzymes with UNG and an optimized dUTP-containing buffer system, enabling robust amplification of target genes in inhibitor-containing samples while effectively preventing false-positive results caused by residual PCR products or aerosol contamination. After lyophilization, the reagent maintains good cake appearance and product stability.

Components

1. 12.5× SensiDircet RTase/UNG Mix (with dNTPs) (DG) (000830)
2. 5× SensiDirect RT Buffer (DG) (000814)
3. 2× Lyoprotectant (Optional)

Storage

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

Reaction Conditions

FAST PCR Protocol			
Step	Temp.	Time	Cycles
Reverse Transcription	50℃	3-5 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 (DG) enzyme contained in this product is active at room temperature and is inactivated during reverse transcription.

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
5× SensiDirect RT Buffer (DG) (000814)	5 μL	10 μL	1×
12.5×SensiDircet RTase/UNG Mix (with dNTPs) (DG) (000830)	2 μL	4 μL	1×
2× Lyoprotectant[1]	12.5 μL	25 μL	1×
25× Primer-Probe Mix[2,3]	1 μL	2 μL	1×
Sample[4]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. This is a lyophilization-compatible system. If lyophilization is not required, 2× Lyoprotectant can be added optionally. If a lyophilized product is intended, 2× Lyoprotectant must be included during liquid-stage performance verification to ensure consistency with the composition and performance of the lyophilized system.

2. When using the standard PCR program, a final primer concentration of 0.2 μM usually gives good results. If reaction performance is suboptimal, the primer concentration can be adjusted within 0.2-1 μM. Probe concentration is usually optimized within 0.1-0.3 μM. Gradient experiments can be performed to determine the optimal primer and probe combination.

3. When using the fast PCR program, appropriately increasing primer and probe concentrations may improve amplification performance. The primer-to-probe ratio should be optimized accordingly.

4. Different types of biological samples contain different inhibitors, inhibitor levels, and target-gene copy numbers. Different sample preservation media (such as swab transport media) may also affect amplification differently. Therefore, the optimal sample input volume should be determined according to the sample type, required detection sensitivity, and actual application conditions. If necessary, samples can first be diluted with nuclease-free water or TE Buffer before addition. Recommended sample input volumes are as follows:

Sample Type	Input Volume in 50 μL System	Maximum Sample Proportion
Anticoagulated Whole Blood	2.5 μL	5%
Throat Swab	10 μL	20%
Saliva	10 μL	20%

Formulation for Lyophilization

Components	25 μL System
5× SensiDirect RT Buffer (DG) (000814)	5 μL
12.5×SensiDircet RTase/UNG Mix (with dNTPs) (DG) (000830)	2 μL
2× Lyoprotectant	12.5 μL
25× Primer-Probe Mix	1 μL
ddH_2O	To 21-25 μL

For lyophilization formulations in other reaction volumes, please contact us.

Lyophilization Conditions

Step	Temperature	Time	Condition	Pressure
Pre-freezing	-50℃	180 min	Hold	1 atm
Primary Drying	-40℃	30 min	Heating	Maximum Vacuum
	-40℃	480 min	Hold	
	-30℃	30 min	Heating	
	-30℃	240 min	Hold	
	-20℃	30 min	Heating	
	-20℃	180 min	Hold	
Secondary Drying	10℃	60 min	Heating	Maximum Vacuum
	10℃	120 min	Hold	
	30℃	30 min	Heating	
	30℃	240 min	Hold	

1. This lyophilization process is an in-situ lyophilization process for a 25 μL system. If freeze-dried beads or in-situ lyophilization of other reaction volumes is required, please contact us.

2. The above lyophilization process is for reference only. Parameters may vary with product type and freeze dryer model, and can be adjusted according to actual conditions.

3. Different lyophilization processes may be suitable for different batch sizes. When used for large-scale production, sufficient testing and verification must be performed.

Protocol for Lyophilized Powder

1. Briefly centrifuge the lyophilized powder.
2. Add the template to the lyophilized powder, then add an appropriate amount of water to bring the total volume to 25 μL.
3. Mix well, centrifuge briefly, and run the reaction.

*Biori CM-8 or CM-8 Plus Centrifugal Mixer is recommended for standardized mixing and centrifugation.

Quality Control

1. Functional testing: sensitivity, specificity, and reproducibility of RT-qPCR.
2. No exogenous nuclease activity and no exogenous endonuclease or exonuclease contamination.

Technical Notes

1. The amplification rate of the DNA polymerase contained in this product is not less than 1 kb/10 s. Different PCR instruments vary substantially in heating/cooling rates, temperature control modes, and heat transfer efficiency. It is recommended to optimize reaction conditions according to the specific fast PCR instrument used.
2. If problems such as low fluorescence signals or obvious amplification inhibition occur, it is recommended to reduce the sample input volume or dilute the sample before addition.
3. Blood, saliva, and throat swab samples should be collected according to standard clinical operating procedures. To avoid nucleic acid degradation, freshly collected samples are recommended.
4. For primers with low annealing temperatures or amplicons longer than 200 bp, a three-step program is recommended.
5. Different target genes vary in dUTP utilization efficiency and sensitivity to UNG. If the UNG system reduces detection sensitivity, the reaction system should be adjusted and optimized accordingly. For technical support, please contact us.
6. Use dedicated work areas and pipettes before and after amplification. Wear gloves during operation and change them frequently. Do not open reaction tubes after PCR is completed in order to minimize sample contamination from PCR products.