Cat.:FMD5091-DB

Sensi Direct Lyo-Ready All-in-One qPCR Master Mix with UNG V01

Product Description

Sensi Direct Lyo-Ready All-in-One qPCR Master Mix with UNG is a specialized reagent developed for lyophilization processes, enabling direct target gene amplification and detection via fluorescent quantitative PCR (TaqMan probe method) without the need for nucleic acid extraction and purification. This product exhibits strong tolerance to inhibitors, allowing direct DNA amplification and detection from various samples including anticoagulated whole blood, plasma, serum, saliva, and swab specimens without nucleic acid purification. The reagent incorporates an inhibition-resistant amplification enzyme and uracil-N-glycosylase (UNG) in a proprietary buffer system optimized with dUTP. This formulation not only ensures robust amplification of target genes in inhibitor-containing samples but also effectively prevents false-positive reactions caused by PCR product carryover and aerosol contamination. Furthermore, the lyophilized reagent demonstrates excellent cake morphology and product stability.

Components

1. 25×SensiDirect Enzyme/UNG Mix (with dNTPs) (DG)
2. 5×SensiDirect Buffer (DG)
3. 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×SensiDirect Buffer (DG)	5 μL	10 μL	1×
25×SensiDirect Enzyme/UNG Mix (with dNTPs) (DG)	1 μL	2 μL	1×
4×AcuGenix™ Lyophilization Protectant-9 [1]	6.25 μL	12.5 μL	1×
25×Primer-Probe Mix [2]	1 μL	2 μL	1×
Template DNA [3]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. This system is lyophilizable. When customers use this system without the need for lyophilization, the 4×AcuGenix™ Lyophilization Protectant-9 may be added optionally. If lyophilized products are required, the 4×AcuGenix™ Lyophilization Protectant-9 must be added during product performance verification at the liquid reagent stage to ensure consistency in composition and performance with the lyophilized system.
2. Usually, a final primer concentration of 0.2 μM can yield good results, when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. Typically, probe optimization is performed in the range of 0.1-0.3 μM. Concentration gradient experiments can be conducted to find the optimal combination of primers and probes.
3. 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 Program			
Procedure	Temp	Time	Cycle
Digestion	50℃	2 min	1
Hot Start	95℃	1-5 min	1
Denaturation	95℃	10-20 s	40-50
Annealing and Extension	56-64℃	20-60 s	

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Prepare Reaction Mix for lyophilization

Composition	Volume per Reaction(25 μL)
5×Superstart Premix plus-UNG (Mg^{2+} free) (DG)	5 μL
250 mM $MgCl_2$	0.45 μL
4×AcuGenix™ Lyophilization Protectant-9	6.25 μL
25×Primer-Probe Mix	1 μL
ddH_2O	To 18-20 μL

*For other lyophilization system formulations, please inquire separately.

Lyophilization conditions

Procedure	Temp.	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 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. Different target genes vary in their utilization efficiency of dUTP and sensitivity to UNG. Therefore, if the use of a UNG system results in reduced detection sensitivity, the reaction system should be adjusted and optimized. For technical support, 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.