Cat.:MD2104-014429

2.5×AcuGenix™ Sensi Direct One-Step Probe RT-qPCR Master Mix with UNG-014429

V01

Product Description

AcuGenix™ Sensi Direct One-Step Probe RT-qPCR Master Mix with UNG-014429 is a specialized reagent for direct one-step RT-qPCR detection of target genes using a fluorescence probe assay, eliminating the need for sample nucleic acid extraction and purification. The product provides strong inhibitor tolerance and enables direct one-step RT-qPCR amplification and detection from anticoagulated whole blood, plasma, serum, throat swabs, saliva, and other sample types without nucleic acid extraction or purification. This reagent uses a temperature-sensitive UNG (TS-UNG) enzyme and an optimized dUTP-containing buffer system to effectively prevent false-positive reactions caused by carryover PCR products and aerosol contamination.

Components

2.5×AcuGenix™ Sensi Direct One-Step Probe RT-qPCR Master Mix with UNG-014429

*This reagent contains a rapid-amplification DNA polymerase, RTase, TS-UNG, PCR Buffer, $MgCl_2$, dNTPs, stabilizers, and other components.

Storage

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

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Conc.
2.5×AcuGenix™ Sensi Direct One-Step Probe RT-qPCR Master Mix with UNG-014429	10 μL	20 μL	1×
25× Primer-Probe Mix[1]	1 μL	2 μL	1×
Template RNA[2]	—	—	—
ddH_2O	To 25 μL	To 50 μL	—

1. 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. Concentration-gradient experiments can be performed to determine the optimal primer and probe combination.
2. Different biological sample types contain different types and levels of inhibitors and different copy numbers of the target gene. Different sample preservation media, such as swab preservation media, may also affect amplification. Therefore, determine the optimal sample input volume based on the sample type, required detection sensitivity, and other practical factors. If necessary, dilute the sample with nuclease-free water or TE Buffer before addition. Recommended input volumes are shown below:

Sample Type	Input Volume in a 50 μL Reaction	Maximum Sample Proportion
Anticoagulated Whole Blood	2.5 μL	5%
Plasma	15 μL	30%
Serum	15 μL	30%
Throat Swab	10 μL	20%
Saliva	10 μL	20%

Reaction Programe

Standard PCR Protocol			
Step	Temperature	Duration	Cycles
Reverse transcriptio n	50°C	10-20 min	1
Hot-start	95°C	1-5 min	1
Denaturatio n	95°C	10-20 s	40-50
Annealing/ Extension	56-64°C	20-60 s	

FAST PCR Protocol			
Step	Temperature	Duration	Cycles
Reverse transcription	50°C	5 min	1
Hot-start	95°C	30 s	1
Denaturation	95°C	1-3 s	40-45
Annealing/ Extension	56-64°C	3-20 s	

*The temperature-sensitive TS-UNG enzyme contained in this product is active at room temperature and is inactivated during reverse transcription.

Quality Control

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

Technical Notes

1. This system commonly uses 50℃ for reverse transcription. The reverse-transcription temperature can be optimized within 42-55℃. The product is suitable for multiplex probe-based real-time RT-PCR assays.
2. If low fluorescence signals or obvious amplification inhibition occurs, reduce the sample input volume or dilute the sample before addition. Alternatively, while keeping the sample input volume unchanged, increasing the reaction volume to 60-100 μL may improve performance.
3. Whole-blood, saliva, throat-swab, and other samples should be collected according to standard clinical operating procedures. Freshly collected samples are recommended to avoid nucleic acid degradation.
4. A three-step program is recommended for primers with low annealing temperatures or for amplification of fragments longer than 200 bp.
5. Different target genes vary in dUTP utilization efficiency and sensitivity to UNG. If use of the UNG system reduces detection sensitivity, adjust and optimize the reaction system accordingly. For technical support, please contact us.
6. Use dedicated work areas and pipettes before and after amplification. Wear gloves and change them frequently. Do not open reaction tubes after PCR is complete to minimize sample contamination by PCR products.