Cat.:MD2091

Sensi Direct All-in-One qPCR Master Mix with UNG

V01

Product Description

Sensi Direct All-in-One qPCR Master Mix with UNG is a specialized qPCR reagent designed for direct quantitative detection of target genes using a probe-based fluorescence detection, bypassing the nucleic acid extraction and purification steps. This reagent exhibits high inhibitor tolerance, enabling direct qPCR amplification from anticoagulated whole blood, plasma, serum, nasopharyngeal swabs, and saliva without prior nucleic acid extraction. The reagent incorporates a UNG enzyme combined with an optimized dUTP-containing buffer system, effectively preventing false-positive amplification caused by PCR product carryover and aerosol contamination.

Components

2×SensiDirect Premix-UNG (Probe qPCR)

*This reagent already contains hot-start DNA polymerase, UNG, PCR Buffer, $MgCl_2$, dNTPs, and stabilizers.

Storage

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

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
2×SensiDirect Premix-UNG (Probe qPCR)	12.5 μL	25 μL	1×
25×Primer-Probe Mix [1]	1 μL	2 μL	1×
Sample [2]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. The final concentration of primers is usually 0.2 μM for better results. when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. The probe concentration is usually optimized in the range of 0.1-0.3 μM. Concentration gradient experiments can be performed to find the best combination of primers and probes.
2. Biological sample types vary in their inhibitor composition, inhibitor concentration, and target gene copy number. The effect of sample storage media (e.g., swab transport media) on amplification also differs. The optimal sample input volume should be determined according to sample type and required detection sensitivity. When necessary, samples may be pre-diluted with nuclease-free water or TE buffer. Recommended volumes for a 50 μL reaction: anticoagulated whole blood 2.5 μL (5%), plasma 10 μL (30%), serum 10 μL (30%), nasopharyngeal swab 10 μL (20%), saliva 10 μL (20%).

Reaction Programe

Standard PCR Program			
Procedure	Temp	Time	Cycle
UNG treatment	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	

Quality Control

1. Functional testin: sensitivity, specificity, and reproducibility of qPCR.
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.