RobuDirect Probe qPCR Premix plus-UNG

Cat.No.:HYA425

Product Description

RobuDirect Probe qPCR Premix plus-UNG is a specialized reagent designed for direct fluorescent quantitative (probe-based) DNA amplification detection of target genes, eliminating the need for nucleic acid extraction and purification from samples. It exhibits strong inhibitor tolerance, enabling direct DNA amplification detection from samples such as anticoagulated whole blood, plasma, serum, throat swabs, and saliva without prior nucleic acid extraction and purification. Formulated with a mixture of inhibition-resistant amplification enzyme and UNG enzyme, along with an optimized Buffer system containing dUTP, this reagent not only achieves efficient amplification of target genes in inhibitor-containing samples but also effectively prevents false-positive reactions caused by PCR product carryover and aerosol contamination.

Components

2×RobuDirect Probe qPCR Premix plus-UNG

*This reagent contains hot start enzyme, UNG, PCR Buffer, $MgCl_2$, dNTPs, stabilizer and other ingredients.

Storage

Store at -20℃ for long-term storage; stable for 3 months at 4℃. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Instruction

1. Reaction system

Components	25uL system	50uL system
2×RobuDirect Probe qPCR Premix plus-UNG	12.5 μL	25 μL
25×Primer-Probe Mix*	1 μL	2 μL
Template DNA**	--	--
ddH_2O	Up to 25 uL	Up to 50 uL

*Typically, a final primer concentration of 0.2 μM yields satisfactory results; if the reaction performance is unsatisfactory, the primer concentration can be adjusted within the range of 0.2–1 μM.

Generally, the probe concentration is optimized within the range of 0.1–0.3 μM. Concentration gradient experiments can be performed to identify the optimal combination of primers and probes. **The types, contents of inhibitors, and copy numbers of target genes vary among different types of biological samples; variations also exist in the impact of different types of sample preservation solutions (e.g., swab preservation solutions) on amplification reactions. Therefore, the optimal sample volume in the reaction system should be determined based on actual conditions such as sample type and detection sensitivity requirements. If necessary, the sample can be diluted with nuclease-free water or TE Buffer before adding it to the reaction system. Recommended sample volumes are as follows:

Sample	50uL system	Maximum loading ratio
whole blood	2.5 μL	5%
Plasma	10 μL	30%
Serum	10 μL	30%
Throat swab	10 μL	30%

2. Reaction program

General PCR program			
Step	**Temperature**	**Time**	**Cycles**
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	

Quality control

1. Functional Testing: qPCR sensitivity, specificity, and repeatability.
2. Free of extraneous nuclease activity, as well as extraneous endonuclease and exonuclease contamination.

Technical description

1. RobuDirect Probe qPCR Premix plus-UNG features a 1–5 minute hot start and is compatible with multiplex real-time probe-based PCR reactions.
2. If issues such as low fluorescence values or significant amplification inhibition occur, it is recommended to reduce the sample volume or dilute the sample before adding it to the reaction

system; alternatively, the problem can be improved by increasing the reaction volume (60–100 μL) while keeping the sample volume unchanged.

3. The collection of samples such as blood and throat swabs mentioned above shall be performed in accordance with clinical standard operating procedures (SOPs); to avoid nucleic acid degradation, freshly collected samples are recommended.
4. For primers with low annealing temperatures or amplification of long fragments exceeding 200 bp, the three-step PCR protocol is recommended.
5. Different target genes vary in their dUTP utilization efficiency and sensitivity to UNG enzyme. Therefore, if the use of the UNG system leads to decreased detection sensitivity, the reaction system should be adjusted and optimized accordingly.
6. Use dedicated areas and pipettes for pre- and post-amplification steps. Operate with gloves and replace them frequently. Do not open the reaction tubes after PCR completion to minimize contamination of samples by PCR amplicons.
7. For Research Use Only !