# RobuDirect Probe RT-qPCR Premix plus-UNG

**Cat.No.:HYA439**

## Product Description

RobuDirect Probe RT-qPCR Premix plus-UNG is a dedicated reagent that eliminates the need for nucleic acid extraction and purification from samples, enabling direct one-step (One-Step) RT-PCR quantitative fluorescence detection of target genes (probe-based method). Boasting exceptional inhibitor tolerance, this reagent allows direct one-step RT-PCR amplification and detection of anticoagulated whole blood, plasma, serum, throat swabs, saliva, and other samples without prior nucleic acid extraction and purification. Formulated with TS-UNG enzyme and an optimized buffer system containing dUTP, it effectively prevents false positive reactions caused by residual PCR products and aerosol contamination.

## Components

1. 10×RobuDirect RTase/UNG Mix
2. 2×RobuDirect RT Premix Buffer (dUTP)

## Storage

Store at -20±5℃; mix well before use and avoid repeated freeze-thaw cycles.

## Instructions

### 1. qRT-PCR Reaction System Preparation

| Components | 25 uL Volume | 50 uL Volume | Final Concentration |
|---|---|---|---|
| 2×RobuDirect RT Premix Buffer (dUTP) | 12.5 μL | 25 μL | 1× |
| 10×RobuDirect RTase/UNG Mix | 2.5 μL | 5 μL | 1× |
| 25×Primer-Probe Mix* | 1 μL | 2 μL | 1× |
| Template RNA** | —— | —— | —— |
| $ddH_2O$ | To 25 μL | To 50 μL | —— |

*Typically, a final primer concentration of 0.2 μM yields optimal results; if 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 between 0.1～0.3 μM. A concentration gradient experiment is recommended to find the optimal combination of primer and probe.

*Different types of biological samples vary in the type and content of inhibitors, as well as the copy number of target genes; the impact of different sample preservation solutions (e.g., swab preservation solution) on the amplification reaction also differs. Therefore, the optimal sample volume in the reaction system should be determined based on actual conditions such as sample type and required detection sensitivity. If necessary, the sample can be diluted with nuclease-free water or TE Buffer before loading. Recommended sample volumes are as follows:

| Sample type | Adding 50 μL of reaction system | Maximum sampling ratio |
|---|---|---|
| AWB | 2.5 μL | 5% |
| Plasma | 10 μL | 30% |
| Serum | 10 μL | 30% |
| Throat swab | 10 μL | 20% |
| Saliva | 10 μL | 20% |

**2. Reaction program**

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Reverse transcription | 50℃ | 10-20 min | 1 |
| Pre-denaturation | 95℃ | 1-5 min | 1 |
| Denaturation | 95 ℃ | 10-20 s | 40-50 |
| Annealing and Extension | 56~64℃ | 20-60 s | |

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Reverse transcription | 50℃ | 5 min | 1 |
| Pre-denaturation | 95℃ | 30 s | 1 |
| Denaturation | 95 ℃ | 1-3 s | 40-45 |
| Annealing and Extension | 56~64℃ | 3-20 s | |

* The temperature-sensitive TS-UNG enzyme contained in this product can perform digestion at room temperature and become inactive during the reverse transcription process.

## Quality Control

1. Function detection: sensitivity, specificity and repeatability of qRT-PCR.
2. No exogenous nuclease activity: no exogenous endonuclease and exonuclease pollution.

## Technical Information

1. This system commonly uses 50℃ for reverse transcription, but the temperature can be optimized within the range of 42℃ to 55℃. Depending on the reaction characteristics, the reverse transcription time can be optimized within 5 to 30 minutes. It is suitable for multiplex quantitative RT-PCR reactions.
2. If problems such as excessively low fluorescence values or significant amplification inhibition occur, it is recommended to reduce the sample volume or dilute the sample before adding it. Alternatively, while maintaining the same sample volume, increasing the reaction volume (60-100 μL) can improve the situation.
3. The collection of blood, saliva, and throat swabs should be performed according to clinical standard operating procedures. To avoid nucleic acid degradation, freshly collected samples can be used.
4. For primers with low annealing temperatures or amplification of fragments longer than 200 bp, a three-step method is recommended.
5. Different genes to be amplified have varying utilization efficiency of dUTP and sensitivity to UNG enzymes. Therefore, if using the UNG system leads to a decrease in detection sensitivity, the reaction system should be adjusted and optimized. Please contact our company for technical support.
6. Please use dedicated regions and pipettes before and after amplification, wear gloves during operation, and change gloves frequently. Do not open the reaction tube after the PCR reaction is complete to minimize PCR product contamination of the sample.
7. For research use only！