

Product Description

AcuGenix™ RT Probe qPCR Master Mix with UNG (Universal ROX) is a specialized reagent designed for probe-based real-time PCR qualitative and quantitative analysis. This product contains a thermostable reverse transcriptase with eliminated RNase H activity, a novel hot-start DNA polymerase, and thermo-labile uracil-N-glycosylase (TS-UNG) enzyme. This product adopts optimally formulated qPCR buffer and UNG/dUTP anti-contamination system, which greatly enhances the amplification efficiency and detection sensitivity of qPCR reaction. It can generate ideal standard curves within a wide quantification range to achieve accurate quantification, and effectively prevent false positive amplification induced by PCR product residual carryover and aerosol contamination. Matched with 4x gDNA Wiper, it can completely remove residual genomic DNA contamination in RNA templates.

Containing universal ROX Reference Dye, this premix is compatible with all qPCR instruments without the need to adjust ROX concentrations for different instruments. PCR amplification can be performed simply by adding primers and template during reaction setup.

Components

| Components | BR1F202-01 100 T (20 µL/T) | BR1F202-02 500 T (20 µL/T) |
|--|-------------------------------|-------------------------------|
| 4xAcuGenix™ RT Probe qPCR Master Mix with UNG (Universal ROX)* | 500 µL | 2×1 mL+0.5 mL |
| 4xgDNA Wiper Mix | 500 µL | 2×1 mL+0.5 mL |

*This reagent contains Reverse Transcriptase, DNA polymerase, TS-UNG enzyme, PCR Buffer, dNTPs, ROX Reference Dye, etc.

Storage

Store at -20±5°C, protected from light.

Notes

- 1.For Research Use Only. Not for use in diagnostic procedures.
- 2.Please prepare the reaction system in a super-clean bench. It is recommended to use a special pipette and tips with a filter element during the preparation process. Operators should wear masks and disposable gloves and change gloves frequently.
- 3.Mix well before use and avoid repeated freeze-thaw cycles. Repeated freeze-thawing may degrade the performance of the product. If the amount used each time is small, dispensing of the reagent is recommended.
- 4.This system typically conducts reverse transcription reactions at 50°C. Reverse transcription temperature can be optimized within the range of 42°C to 55°C, and reverse transcription time can be adjusted between 5 and 30 minutes based on specific reaction characteristics.
- 5.Because this product contains ROX Reference Dye, strong light should be avoided when preparing reaction system.
- 6.The Neoscript RTase employed in this system is genetically engineered from M-MLV reverse transcriptase. It features enhanced temperature tolerance and a higher optimal reverse transcription temperature, delivering significantly improved reverse transcription efficiency for RNA templates with complex secondary structures.
- 7.This system demonstrates exceptional stability and broad applicability, making it highly suitable for the detection of complex RNA templates derived from viruses and tissue extracts. It provides remarkably consistent amplification performance for extremely low-concentration templates, and is ideal for use in high-sensitivity molecular diagnostic reagents.
- 8.For primers with low annealing temperature or long fragments over 200 bp, three-step method is recommended.
- 9.DO NOT open reaction tubes after completion of the PCR reaction to minimize PCR product carryover contamination of samples.

Prepare Reaction Mix

1.Genomic DNA Removal from RNA Templates (Optional)

| Components | Volume per Reaction |
|-------------------------------|---------------------|
| 4×gDNA Wiper Mix | 5 µL |
| Template RNA | 10 pg-1 µg* |
| RNase-free ddH ₂ O | To 20 µL |

*Add an appropriate amount of RNA template according to experimental requirements.

2.Mix thoroughly by pipetting up and down, then incubate at 42°C for 2 minutes (temperature control can be performed using a thermal cycler).

3.Take a new RNase-free microcentrifuge tube, add the following components to a final volume of 20 µL (prepare on ice), and mix gently.

| Components | Volume per Reaction |
|---|---------------------|
| 4×AcuGenix™ RT Probe qPCR Master Mix with UNG (Universal ROX) | 5 µL |
| 20×Primer Mix | 1 µL* |
| Template RNA | 10 pg-1 µg |
| RNase-free ddH ₂ O | To 20 µL |

*The optimal final primer concentration is usually 0.2 µM for good results. For poor reaction performance, the primer concentration can be adjusted within the range of 0.2-1 µM. The probe concentration is generally optimized from 0.1 to 0.3 µM. Concentration gradient experiments can be carried out to find the optimal combination of primers and probes.

Reaction Program

| Steps | Temp | Time | Cycles |
|---------------------------------------|---------|--------|--------|
| Reverse transcription | 50°C | 10 min | 1 |
| Initial denaturation ¹ | 95°C | 1 min | 1 |
| Degeneration ² | 95°C | 10 s | 40 |
| Annealing and Elongation ³ | 56-64°C | 30 s | |

1.The pre-denaturation conditions are suitable for most amplification reactions. For templates with complex structures, the pre-denaturation time can be extended to 3 minutes to enhance the pre-denaturation effect.

2.For fragments longer than 200 bp, it is recommended to appropriately prolong the extension time; the annealing and extension temperature should be adjusted according to the T_m value of the designed primers.

3.Set signal acquisition at marked position.