AcuGenix™ RT qPCR Master Mix with UNG (Universal ROX)

Cat.:BR1F102

V01

Product Description

AcuGenix™ RT qPCR Master Mix with UNG (Universal ROX) is a specialized reagent designed for Real-Time PCR using fluorescent dye intercalating fluorescence method. 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 4×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	BR1F102-01 100 rxns (20 μL/rxn)	BR1F102-02 500 rxns (20 μL/rxn)
4×AcuGenix™ RT qPCR Master Mix with UNG (Universal ROX)*	500 μL	2×1mL+0.5 mL
4×gDNA Wiper Mix	500 μL	2×1mL+0.5 mL

*This reagent contains Reverse Transcriptase, DNA polymerase, TS-UNG enzyme, PCR Buffer, dNTPs, fluorescent dye, etc.

Storage

Store at -20±5℃, 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 fluorescent 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.

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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_2O	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 qPCR Master Mix with UNG (Universal ROX)	5 μL
20×Primer Mix	1 μL*
Template RNA	10 pg-1 μg
RNase-free ddH_2O	To 20 μL

*When amplifying with conventional PCR procedures, 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.

Reaction Program

Steps	Temp	Time	Cycles
Reverse transcription	50℃	10 min	1
Initial denaturation[1]	95℃	1 min	1
Degeneration[2]	95℃	10 s	40
*Annealing and Elongation[3]	56-64℃	30 s	
*Melting curve analysis[4]			

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 Tm value of the designed primers.

3.Melting curve analysis: Please set the program recommended by the fluorescence quantitative PCR instrument used.

4.* Set signal acquisition at marked position.