Cat.:BR1D301

AcuGenix™ 1st cDNA Synthesis Premix for qPCR (＋gDNA wiper) V02

Product Description

AcuGenix™ 1st cDNA Synthesis Premix for qPCR (＋gDNA wiper) contains all reagents required for first-strand cDNA synthesis and is suitable for two-step qRT-PCR detection. 4×gDNA Wiper Mix can thoroughly eliminate residual genomic DNA contamination in RNA templates, ensuring more accurate quantification results. It also simplifies qPCR primer design, eliminating the need for intron-spanning primers. The 5×Neoscript 1st cDNA Synthesis Premix is an efficient and convenient first-strand cDNA synthesis master mix. The reverse transcription reaction can be performed simply by adding the RNA template and nuclease-free water.

cDNA synthesized with this product is compatible with both fluorescent dye and probe-based qPCR platforms. Corresponding supporting reagents can be selected according to experimental purposes for high-performance gene expression analysis.

Components

Components	BR1D301-01 100 T (20 μL/T)	BR1D301-02 500 T (20 μL/T)
4×gDNA Wiper Mix	40 μL	400 μL
5×Neoscript 1st cDNA Synthesis Premix[1]	40 μL	400 μL
5×No RT Control Mix[2]	4 μL	40 μL
RNase-free ddH_2O	200 μL	2×1mL

1.This reagent contains components that inhibit DNase activity. Samples treated with 4×gDNA Wiper Mix can be directly subjected to reverse transcription for cDNA synthesis, enabling rapid completion of the entire process from genomic DNA removal to cDNA synthesis. It contains Random 6 mers, Oligo (dT)18 Primer and dNTPs, allowing efficient and uniform synthesis of various cDNAs.

2.Excluding Neoscript RTase, this reagent shares the same composition as 5×Neoscript 1st cDNA Synthesis Premix, and is used for preparing No RT control reactions.

Storage

Store at -20±5℃.

Notes

1.For Research Use Only. Not for use in diagnostic procedures.

2.Mix thoroughly before use and avoid repeated freeze-thaw cycles.

3.For long-fragment amplification, to prevent destruction of the cDNA structure, incubation at 70 °C for 15 min is recommended for enzyme inactivation.

4.The addition of RNase Inhibitor can effectively inhibit RNA template degradation caused by nucleases introduced during RNA extraction.

5.High-purity and intact RNA templates are critical for reverse transcription to generate high-quality and full-length cDNA strands. The addition of gDNA Wiper maximizes the accuracy and reproducibility of qPCR results.

Prepare RT Reaction Mix

1.Reaction system setup (prepare on ice).

Components	Volume per Reaction
4×gDNA Wiper Mix	5 μL
Template RNA	10 pg -1 μg*
RNase-free ddH_2O	To 16 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.Add the following components to the above system to a final volume of 20 μL (prepare on ice), and mix gently.

Components	Volume per Reaction
5×Neoscript 1st cDNA Synthesis Premix	4 μL

4.Reverse transcription program: 50 ℃ for 15 min; 85 ℃ for 5 s.

*For templates with complex secondary structures or high-GC regions, the reaction temperature can be appropriately increased.

5.The reverse transcription product can be directly used for conventional PCR and real-time fluorescent quantitative PCR, or stored at -20 ℃.

Prepare Reaction Mix

The following is the operational procedure for subsequent qPCR detection on a real-time fluorescent quantitative PCR instrument using AcuGenix™ FastAmpli qPCR Master Mix (Universal ROX) (Cat.: BR1E102). If used with other Biori qPCR products, please refer to the corresponding product manual.

Components	Volume per Reaction
2×AcuGenix™ FastAmpli qPCR Master Mix (Universal ROX)	10 μL
Forward Primer (10 μM)	0.4 μL
Reverse Primer (10 μM)	0.4 μL
cDNA*	-
RNase-free ddH_2O	To 20 μL

*It is recommended to dilute cDNA with RNase-free water, and the volume of added cDNA shall not exceed 1/10 of the total qPCR reaction system volume.

Reaction Program

Conventional PCR procedures			
Steps	Temp	Time	Cycles
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]			

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

1.The pre-denaturation conditions are applicable to most amplification reactions. The conventional PCR procedure uses 1 min, while the fast PCR procedure can be set to a minimum of 30 s. For templates with complex structures, the pre-denaturation time can be extended to 3 minutes to improve the pre-denaturation effect.

2.Denaturation: 10 s for the conventional PCR procedure, and a minimum of 1 s for the fast PCR procedure.

3.Annealing/Extension: 30 s for the conventional PCR procedure. For the fast PCR procedure, the extension time can be set to 10 s for amplicons within 200 bp. For products longer than 200 bp, it is recommended to appropriately extend the extension time or adopt a three-step protocol. The annealing and extension temperature shall be adjusted according to the Tm value of the designed primers.

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

5.* Set signal acquisition at marked position.