HiScript IV All-in-One RT Red SuperMix for qPCR (+gDNA Wiper)

RT433

Version 26.1

Product Description

HiScript IV All-in-One RT Red SuperMix for qPCR (+gDNA Wiper) is an upgraded all-in-one reverse transcription reagent designed for qPCR applications, enabling visual confirmation of pipetting. All components required for genomic DNA removal and RNA reverse transcription are pre-mixed in a single tube, requiring only the addition of RNA template and ddH_2O. Pipetting can be visually confirmed during cDNA synthesis. The optimized red tracking dye, with no interference in fluorescent signal acquisition for downstream dye- or probe-based qPCR assays, ensures amplification sensitivity and plateau-phase stability.

Components

	Components	RT433-01 100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	4 × HiScript IV All-in-One qRT Red SuperMix*	500 μl

* Includes Reverse Transcriptase, DNase, RNase Inhibitor, dNTP Mix, Random Primers/Oligo(dT)$_{20}$VN Primer Mix, red tracking dye, etc.

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

The SuperMix is broadly applicable for reverse transcription of animal, plant and microbial RNA. The resulting cDNA is compatible with dye- and probe-based qPCR and conventional PCR.

Self-prepared Materials

Materials

RNase-free centrifuge tube (1.5 ml), RNase-free PCR tube (0.2 ml), RNase-free tips, pipette, ice box

Equipment

PCR instrument

qPCR Reagents Selection Guide

SupRealQ Ultra Hunter SYBR qPCR Master Mix (U^+) (Vazyme #Q713)

Notes

1. RNA Input: In a 20 μl reverse transcription reaction, ≤1 μg total RNA is recommended; for low-expression targets, which can be increased to a maximum of 5 μg for low-expression targets; exceeding this maximum may result in qPCR detection outside the linear range.
2. Long cDNA Synthesis: With RNA template denaturation (refer to “Downstream Application: PCR (Optional)”), efficient synthesis of cDNA up to 5 kb can be achieved. For downstream long-fragment PCR amplification, use in combination with HiScript IV 1st Strand cDNA Synthesis Kit (+gDNA Wiper) (Vazyme #R412) is recommended for optimal performance.
3. The cDNA can be used directly as a template for qPCR, with a template volume ≤10% of the total reaction volume.

Experiment Process

◇ Downstream Application: qPCR

1. Reaction System

Mix the following components in an RNase-free centrifuge tube:

Components	Volume	
RNase-free ddH_2O	to 20 μl	☐
4 × HiScript IV All-in-One qRT Red SuperMix	5 μl	■
Template RNA	Total RNA: 1 pg - 1 μg	

Optional: For GC-rich RNA templates or RNA with strong secondary structures, after gentle mixing and brief centrifugation, incubate at 65 °C for 5 min. Chill on ice, briefly centrifuge again and keep on ice for 2 min.

2. Reaction Program

Temperature	Time
50°C	5 min
85°C	5 sec

The product can be used for qPCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage to avoid repeated freeze-thaw cycles of cDNA.

◇ Downstream Application: PCR (Optional)

1. RNA template denaturation

Mix the following components in an RNase-free centrifuge tube:

Components	Volume	
RNase-free ddH_2O	to 15 μl	☐
Template RNA	Total RNA : 1 pg - 1 μg	

Incubate at 65°C for 5 min, immediately transfer to an ice bath for rapid cooling, and keep on ice for 2 min.

2. Prepare the first-strand cDNA synthesis reaction mixture

Mix the following components in an RNase-free centrifuge tube:

Components	Volume	
Mixture of step1	15 μl	
4 × HiScript IV All-in-One qRT Red SuperMix	5 μl	■

Gently mix by pipetting.

3. Reaction Program

Temperature	Time
50°C	15 min
85°C	5 sec

▲ Under standard reaction conditions (15 min), efficient synthesis cDNA up to 5 kb in length. For higher cDNA yield, the reaction time can be extended to 30 min.

The product can be used for qPCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage to avoid repeated freeze-thaw cycles of cDNA.