HiScript III RT SuperMix for qPCR (+gDNA wiper)

R323

Version 23.1

Product Description

HiScript III RT SuperMix for qPCR (+gDNA wiper) is an upgraded version of HiScript II Q RT SuperMix for qPCR (+gDNA wiper), including HiScript III Reverse Transcriptase, a new generation of reverse transcriptase with optimized Buffer. This kit further improves the efficiency of cDNA synthesis, and is suitable for two-step qRT-PCR detection. The 4 × gDNA wiper Mix in the kit can remove residual genomic DNA from the RNA template, ensuring more reliable quantitative results. It simplifies qPCR primer design without the need of spanning an exon-exon junction; 5 × HiScript III qRT SuperMix contains all the components required for the reverse transcription reaction. The reaction can be performed quickly by adding template RNA and RNase-free ddH_2O, and at the same time, the gDNA wiper function is terminated to ensure the integrity of the cDNA. It is compatible with dye-based and probe-based qPCR, enabling high-performance gene expression analysis.

Components

	Components	R323-01 100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	4 × gDNA wiper Mix	400 μl
■	5 × HiScript III qRT SuperMix[a]	400 μl
■	5 × No RT Control Mix[b]	40 μl

a. It contains Buffer, dNTP, HiScript III Reverse Transcriptase, RNase inhibitor, Random primers/Oligo $(dT)_{20}$VN primer Mix.

b. It does not contain HiScript II reverse transcriptase. Other components are the same as 5 × HiScript II qRT SuperMix for the preparation of No RT Control reaction.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for reverse transcription reactions of animal, plant and microbial RNA. The RT product is compatible with dye-based and probe-based qPCR.

Self-prepared Materials

Materials:

- RNase-free centrifuge tube (1.5 ml), RNase-free PCR tube (0.2 ml), RNase-free tips
- Pipette, PCR instrument, ice or ice box

RNA

- High quality RNA is essential for obtaining high quality cDNA. Please verify the RNA integrity by electrophoresis before the experiment.

qPCR Reagent Selection Guide:

- The 1st strand cDNA product can be used as the template for qPCR directly. For PCR, it is recommended that the volume of the template cDNA product should not exceed 1/10 of the total volume of qPCR reaction. AceQ Universal Probe Master Mix V2 (Vazyme #Q513-EN) or Taq Pro Universal SYBR qPCR Master Mix (Vazyme #Q712) can be selected as the qPCR reagent.

Notes

For research use only. Not for use in diagnostic procedures.

1. The 4 × gDNA wiper Mix, 5 × HiScript III qRT SuperMix and 5 × No RT Control Mix contain high concentration of glycerol. Please centrifuge briefly and pipette up and down to mix thoroughly before use.
2. It is recommended to add no more than 1 μg of total RNA to the 20 μl reverse transcription reaction system. If target genes with low expression levels, the amount of total RNA can be up to 5 μg. Otherwise, the amount of RNA added is too high, which may will exceed the linear range of subsequent qPCR.
3. The cDNA products are only suitable for qPCR reactions and not suitable for long-fragment PCR amplification of downstream experiments such as cloning. If necessary, HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme #R312) is recommended.
4. Reverse transcription can be performed directly with 5 × HiScript III qRT SuperMix without the genome removal step, but do not use 4 × gDNA wiper Mix with reagents without genome removal module, as the Mix in the kits without genome removal module does not contain the components to terminate the gDNA wiper reaction, which may affect subsequent qPCR results.

Experiment Process

1. Removal of genomic DNA

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

RNase-free ddH_2O	to 16 μl	☐
4 × gDNA wiper Mix	4 μl	■
Template RNA	Total RNA: 1 pg - 1 μg	

Gently pipette up and down several times to mix thoroughly, then incubate at 42℃ for 2 min.

2. Preparation of reverse transcription reaction mixture

Add 5 × HiScript III qRT SuperMix to the mixture of previous step:

5 × HiScript III qRT SuperMix	4 μl	■
Mixture from Step 1	16 μl	

Gently pipette up and down several times to mix thoroughly.

No RT Control Reaction (Optional)

No RT Control Reaction is a negative control which contains no Reverse Transcriptase and is used to indicate whether there is residual genomic DNA in RNA template.

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

5 × No RT Control Mix	4 μl	■
Mixture from Step 1	16 μl	

Gently pipette up and down several times to mix thoroughly.

3. Reaction Program

37℃*	15 min
85℃	5 sec

* For template with complex secondary structures or high GC content, the temperature can be increased to 50℃, which will benefit the yield.

The product can be used for qPCR immediately or be stored at -20℃ for 6 months. It is recommended to store in aliquots at -70℃ for long term storage. cDNA should avoid repeated freezing and thawing.