HiScript II Q RT SuperMix for qPCR (+gDNA wiper)

R223

Version 22.2

Product Description

HiScript II Reverse Transcriptase is a novel reverse transcriptase obtained by *in vitro* molecular evolution technology, based on M-MLV (RNase H-) Reverse Transcriptase, HiScript II Reverse Transcriptase greatly improves the thermal stability and cDNA synthesis efficiency. The HiScript II Q RT SuperMix for qPCR (+gDNA wiper) is specially designed for two-step qRT-PCR. The residual genomic DNA in RNA template can be removed rapidly with the 4 × gDNA Wiper Mix. The 5 × HiScript qRT SuperMix II contains all necessary components required for reverse transcription. With the addition of template RNA and ddH_2O, the reaction can proceed quickly, meanwhile, gDNA wiper is terminated to ensure the integrity of the cDNA.

This product has been specially optimized for qPCR. With the optimized proportion of Random primers/Oligo $(dT)_{23}VN$ primer mix, cDNA synthesis can start from each region of RNA transcript and have the same reverse transcription efficiency, which ensures the authenticity and reproducibility of qPCR results. The RT product is compatible with dye-based and probe-based qPCR, and can be used with the corresponding reagent according to the experimental purpose to perform high-performance gene expression analysis.

Components

	Components	R223-01 100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	4 × gDNA wiper Mix	400 μl
■	5 × HiScript II qRT SuperMix II[a]	400 μl
■	5 × No RT Control Mix[b]	40 μl

a. It contains Buffer, dNTPs, HiScript II Reverse Transcriptase, RNase inhibitor, and Random primers/Oligo $(dT)_{23}VN$ primer Mix.

▲ Different from the 5 × HiScript II qRT SuperMix in HiScript II Q RT SuperMix for qPCR (Vazyme #R222), they cannot be mixed.

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 reaction 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 box

RNA

- High quality intact RNA is essential to obtain high quality cDNA. Please verify the RNA integrity by electrophoresis before the experiment.

qPCR Reagents Selection Guidance

- The 1st strand cDNA product can be used as the template for qPCR directly. It is recommended that the template volume of cDNA product should be ≤1/10 of the total volume of qPCR system.
- Taq Pro Universal SYBR qPCR Master Mix (Vazyme #Q712) or Taq Pro Multiple Probe qPCR Mix (Vazyme #QN213-EN) 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 II qRT SuperMix II, and 5 × No RT Control Mix contain high concentration of glycerol. Please centrifuge briefly before use and pipette up and down to mix thoroughly.
2. It is recommended to add no more than 1 μg 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. If the volume of the template RNA is more than 2 μl, please make sure that the RNA is dissolved in RNase-free ddH_2O and not in TE.
4. The cDNA is only suitable for qPCR, not for long fragment PCR amplification in downstream experiments such as cloning. If necessary, use HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme #R212) to perform the operation.
5. Reverse transcription can be performed directly with 5 × HiScript II qRT SuperMix II, without the genome removal step, and the results will be comparable to those obtained with HiScript II Q RT SuperMix for qPCR (Vazyme #R222). Please do not use 4 × gDNA wiper Mix with R222. Because 5 × HiScript II qRT SuperMix of R222 can not terminate the reaction of gDNA digestion, which may affect the subsequent qPCR result.

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: 1pg - 1 μg	

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

2. Preparation of RT reaction system

Add 4 μl of 5 × HiScript II qRT SuperMix II to the mixture of Step 1 (16 μl) and mix thoroughly:

5 × HiScript II qRT SuperMix II	4 μl	■
Mixture of Step 1	16 μl	

Gently pipette up and down several times to mix thoroughly.

No RT Control (Optional)

No RT Control refers to the negative control without reverse transcriptase, which is used to detect whether there is residual genomic DNA in the RNA template.

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

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

Gently pipette up and down several times to mix thoroughly.

3. Reaction Program

50℃*	15 min
85℃	5 sec

*For templates with complex secondary structure or high GC content, the temperature can be increased to 55℃, 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.