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

R223

Version 22.1

Product Description

HiScript II Reverse Transcriptase, optimized from M-MLV (RNase H-) Reverse Transcriptase, is a new generation reverse transcriptase with highly improved thermo stability and cDNA synthesis efficiency. The HiScript II Q RT SuperMix for qPCR (+gDNA wiper) is specially designed for 2-step qRT-PCR. The residual genomic DNA in RNA template can be removed rapidly and completely 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 to the greatest extent. The cDNA products are compatible with SYBR Green and probe 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 component in HiScript II Q RT SuperMix for qPCR (Vazyme #R222), it can not be mixed.
b. Except for HiScript II Reverse Transcriptase, other components are the same as 5 × HiScript II qRT SuperMix II, using for No RT control preparation.

Storage

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

Applications

This product is suitable for reverse transcription reaction of animal, plant and microbial RNA, and the products are 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 complete RNA is essential to obtain high quality cDNA. Please verify the RNA integrity by electrophoresis before the experiment.

qPCR Reagents 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 qPCR SYBR Green Master Mix (Vazyme #Q111), AceQ qPCR Probe Master Mix (Vazyme #Q112) or ChamQ SYBR qPCR Master Mix (Vazyme #Q311) can be selected as the qPCR reagent.

Notes

1. The 4 × gDNA wiper, 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 large (more than 2 μl), use RNase-free ddH_2O instead of TE to dissolve total RNA, for the EDTA in TE inhibits the reverse transcription reaction.
4. The cDNA products are only suitable for qPCR reaction, not for long fragment PCR amplification in downstream experiments such as cloning. If necessary, use HiScript II 1st Strand cDNA Synthesis Kit (Vazyme #R211) 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't terminate the reaction of gDNA wiper, which may affect the subsequent qPCR result.

Experiment Process

1. Removal of Genomic DNA

Mix the following components thoroughly in a RNase-free PCR tube:

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

Mix gently with a pipette and 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 from Step 1	16 μl	

Mix gently with a pipette.

No RT Control (Optional)

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

Prepare the following reaction mix in a RNase-free centrifuge tube:

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

Mix gently with a pipette.

3. Reverse transcription

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 products can be used for PCR immediately or be stored at -20℃ for 6 months. However, it is recommended to store in aliquots at -70℃ for long term storage and cDNA should be avoided repeated freezing and thawing.