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

Catalog # R223

Version 7.0

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Introduction

The Vazyme HiScript II Reverse Transcriptase, optimized from M-MLV (RNase H-) Reverse Transcriptase, is a new generation reverse transcriptase with highly improved heat stability and cDNA synthesis efficiency. The residual genomic DNA in RNA template can be removed rapidly and completely with the 4× gDNA Wiper.The HiScript II Q RT SuperMix for qPCR is specially designed for 2-step RT-qPCR. The 5×Mix contains all necessary components needed for reverse transcription, including Buffer, dNTPs, HiScript II Reverse Transcriptase, RNase inhibitor, and Random primers/Oligo-$(dT)_{23}$VN primer mix.

The Vazyme HiScript II Q RT SuperMix for qPCR (+gDNA Wiper) has been specially optimized for qPCR. For example, the ratio of Random primers/Oligo-$(dT)_{23}$VN primer is optimized to enable cDNA synthesis at any region of the template RNA and to ensure the repeatability of qPCR results. The cDNA products are compatible for SYBR- or probe-basded qPCR, such as AceQ qPCR SYBR Green Master Mix (Vazyme, #Q111), ChamQ SYBR qPCR Master Mix (Vazyme, #Q311), ChamQ Color SYBR qPCR Master Mix (Vazyme, #Q411), and AceQ qPCR Probe Master Mix (Vazyme, #Q112).

Contents of Kits

Components	R223-01 100 rxn (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. contains Buffer, dNTPs, HiScript II Reverse Transcriptase, RNase inhibitor, and Random primers/Oligo $(dT)_{23}$VN primer mix.
b. contains no HiScript II Reverse Transcriptase, used for control.

Storage

All components should be stored at -20℃.

Additional Materials Required

RNase-free microtube (1.5 ml) or PCR tube (0.2 ml).
Thermocycler (PCR instrument) or water bath.
Ice bath

Protocol

Note: 1. Use high quality total RNA with high intergrity for reverse transcription.
2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.

1. Removal of Genomic DNA

Mix the following components thoroughly in a RNase-free PCR tube and incubate at 42℃ for 2 min.

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

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

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. Add 4 μl of 5×No RT Control Mix to the mixture of **Step 1** (16 μl) and mix thoroughly.

3. Reverse transcription

50℃*	15 min
85℃	5 sec

Note: * 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 stored at -80℃ and make aliquots to avoid repeated freezing and thawing.

Tips

1. The 4× gDNA Wiper, 5× HiScript II qRT SuperMix II, and 5× No RT Control Mix contain glycerol. Therefore, before pipetting, please collect the liquid by a brief centrifugation.
2. It is recommended that in a 20 μl reverse transcription reaction system, the amount of total RNA is ≤ 1 μg. However, for target genes with low expression levels, the amount of total RNA can be ≤ 5 μg.
3. Use RNase-free water to dissolve total RNA. Don't use TE, for the EDTA in TE inhibits the reverse transcription reaction.
4. The cDNA product can be used for qPCR, and is not suitable for long-fragment PCR and molecular cloning.
5. The 4× gDNA Wiper of this kit is **NOT** compatible for Vazyme HiScript II Q RT SuperMix for qPCR (Vazyme, #R222).