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

Catalog # R233

Version 5.1

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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 Select RT SuperMix for qPCR (+gDNA Wiper) is designed for 2-step RT-qPCR. The 5× Mix contains Buffer, dNTPs, HiScript II Reverse Transcriptase, and RNase inhibitor. Random primers, Oligo-dT primers, and Gene Specific Primers can be used for reverse transcription.

The HiScript II Q Select RT SuperMix for qPCR (+gDNA Wiper) has been specially optimized for qPCR. 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	R233-01 200 rxn (10 μl/rxn)
RNase free ddH_2O	1 ml × 2
4× gDNA Wiper Mix	400 μl
5× HiScript II Select qRT SuperMix II [a]	400 μl
Oligo $(dT)_{18}$ (10 μM)	100 μl
Random hexamers (50 ng/μl)	100 μl
5× Select No RT Control Mix [b]	40 μl

a. contains Buffer, dNTPs, HiScript II Reverse Transcriptase, and RNase inhibitor.
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 8 μl
4× gDNA Wiper Mix	2 μl
Oligo $(dT)_{18}$ (10 μM)	
or Random Hexamers (50 ng/μl)	0.5 μl
or Gene Specific Primers (2 μM)	
Template RNA	Total RNA: 1 pg-500 ng

2. Add 2 μl of 5× HiScript II Select qRT SuperMix II to the mixture of **Step 1** (8 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 2 μl of 5× Select No RT Control Mix to the mixture of **Step 1** (8 μl) and mix thoroughly.

3. Reverse transcription

50℃*	15 min
85℃	2 min

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 Select 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 10 μl reverse transcription reaction system, the amount of total RNA is ≤ 500 ng. However, for target genes with low expression levels, the amount of total RNA can be ≤ 1 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 Select RT SuperMix for qPCR (Vazyme, #R232).