HiScript II Q RT SuperMix for qPCR

Catalog # R222

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 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 primer mix.

The HiScript II Q RT SuperMix for qPCR has been specially optimized for qPCR. For example, the ratio of Random primers/Oligo-dT 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-based 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	R222-01 200 rxn (10 μl/rxn)
RNase-free ddH_2O	1 ml × 2
5× HiScript II qRT SuperMix [a]	400 μl
5× No RT Control Mix [b]	40 μl

a. contains Buffer, dNTP, HiScript II Reverse Transcriptase, RNase inhibitor, and Random primers/Oligo dT 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. Mix the following components in a RNase-free PCR tube for 1st-strand cDNA synthesis:

RNase free ddH_2O	to 10 μl
5× HiScript II qRT SuperMix	2 μl
Template RNA	Total RNA: 1 pg-500 ng

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

RNase free ddH_2O	to 10 μl
5× No RT Control Mix	2 μl
Template RNA	Total RNA: 1 pg-500 ng

2. Reverse transcription (Standard or Fast program).

Standard Program

25℃	10 min
50℃*	30 min
85℃	5 min

Fast Program (used for most RT-qPCR)

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. Both 5× HiScript II qRT SuperMix 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. If the Ct value difference between No RT Control and Experimental Group is < 5, which inidicates that the template RNA has been contaminate by genomic DNA, it is suggested to use HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, #R223) to eliminate genomic DNA in RNA templates.
5. For reverse transcription, the Fast Program is suitable for most RT- qPCR. Generally there is no difference between the results of using Fast Program and that of using Standard Program. However, please switch to Standard Program if the amplification efficiency is poor or the Ct value is too high.