HiScript II Q RT SuperMix for qPCR

R222

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 is specially designed for two-step qRT-PCR. The 5 × HiScript qRT SuperMix contains all components required for reverse transcription. With the addition of template RNA and ddH_2O, the reaction can proceed quickly. The volume of template RNA can be up to 80% of the total volume, which is very suitable for reverse transcription reaction at low concentration of template RNA. 5 × HiScript II qRT SuperMix will not freeze at -20℃.

This product has been specially optimized for qPCR. Oligo $(dT)_{23}VN$ has stronger anchoring ability for poly A^+ mRNA than Oligo $(dT)_{18}$, resulting in higher reverse transcription efficiency. 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	R222-01 100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	5 × HiScript II qRT SuperMix[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.

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 5 × HiScript II qRT SuperMix 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 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. If absolute value of ΔC_T between No RT Control and experimental group was less than 5, it indicated that the RNA template might be contaminated by genomic DNA. For superior cDNA synthesis performance in qPCR applications, we recommend HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme #R223).
5. The cDNA is only suitable for qPCR, not for long fragment PCR amplification in downstream experiments such as cloning. If necessary, HiScript II 1st Strand cDNA Synthesis Kit (Vazyme #R211) can be used to perform the operation.

Experiment Process

1. Preparation of reaction solution for 1st strand cDNA synthesis

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

RNase-free ddH_2O	to 20 μl	☐
5 × HiScript II qRT SuperMix	4 μl	■
Template RNA	Total RNA: 1 pg - 1 μg	

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:

RNase-free ddH_2O	To 20 μl	☐
5 × No RT Control Mix	4 μl	■
Template RNA	Total RNA: 1 pg - 1 μg	

Gently pipette up and down several times to mix thoroughly.

2. 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.