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

R233

Version 22.1

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 Select RT SuperMix for qPCR (+gDNA wiper) is specially designed for two-step qRT-PCR. The 4 × gDNA wiper Mix in the product can rapidly remove the residual genomic DNA contamination and ensure more reliable subsequent quantitative results. 5 × HiScript II Select qRT SuperMix II contains Buffer, dNTP mix, HiScript II Reverse Transcriptase and RNase inhibitor. Oligo $(dT)_{23}VN$, Random primers or Gene Specific Primers (GSP) can be selected as reverse transcription primers according to requirements. Oligo $(dT)_{23}VN$ has stronger anchoring ability to Poly A^+ mRNA than Oligo$(dT)_{18}$, making reverse transcription more efficient. 5 × HiScript II Select qRT SuperMix does not freeze at -20℃, and the reaction proceeds quickly after adding template RNA and primers. The RT product is compatible with dye-based and probe-based qPCR. According to the purpose of the experiment, the corresponding reagents can be selected for high-performance gene expression analysis.

Components

	Components	R233-01 100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	4 × gDNA wiper Mix	400 μl
■	5 × HiScript II Select qRT SuperMix II[a]	400 μl
■	Oligo $(dT)_{23}VN$ (10 μM)	100 μl
■	Random hexamers (50 ng/μl)	100 μl
■	5 × Select No RT Control Mix[b]	40 μl

a. It contains Buffer, dNTP mix, HiScript II Reverse Transcriptase and RNase inhibitor.

▲ Different from the 5 × HiScript II Select qRT SuperMix in HiScript II Q Select RT SuperMix for qPCR (Vazyme #R232), they cannot be mixed.

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

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 Guide

- 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 4 × gDNA wiper Mix, 5 × HiScript II Select qRT SuperMix II, and 5 × Select No RT Control Mix contain high concentration of glycerol. Please centrifuge briefly before use, then 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 more than 2 μl, please make sure that the RNA is dissolved in RNase-free ddH_2O and not in TE.
4. Reverse transcription can be performed directly with 5 × HiScript II Select qRT SuperMix II, without the genome removal step. The results will be comparable to those obtained with HiScript II Q Select RT SuperMix for qPCR (Vazyme #R232). Please do not use 4 × gDNA wiper Mix with R232. Because 5 × HiScript II Select qRT SuperMix of R232 can not terminate the reaction of gDNA digestion, which may affect the subsequent qPCR result.
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 (+gNDA wiper) (Vazyme #R212) can be used to perform the operation.

Experiment Process

1.Removal of Genomic DNA

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

RNase-free ddH_2O	to 16 μl	□
4 × gDNA wiper Mix	4 μl	■
Oligo $(dT)_{23}VN$ (10 μM)	1 μl	■
or Random hexamers (50 ng/μl)	1 μl	■
or Gene Specific Primers (2 μM)	1 μl	
Template RNA	Total RNA: 1 pg - 1 μg	

Gently pipette up and down several times to mix thoroughly. Incubate at 42℃ for 2 min.

2. Preparation of RT reaction system

Add 5 × HiScript II Select qRT SuperMix II to the mixture of Step 1 (16 μl):

5 × HiScript II Select qRT SuperMix II	4 μl	■
Mixture of Step 1	16 μl	

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:

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

Gently pipette up and down several times to mix thoroughly.

3. 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.