HiScript IV RT SuperMix for qPCR (+gDNA wiper)

#Cat: NB-54-0395-01 **Size: 100rxns**

Product Description

HiScript IV RT SuperMix for qPCR (+gDNA wiper) is an upgraded version of HiScript III RT SuperMix for qPCR (+gDNA wiper), including a new generation of HiScript IV Reverse Transcriptase and Buffer optimized for reverse transcription. This kit further improves the synthesis efficiency of cDNA, making it a better choice for reverse transcription of low-input, low-expression or degraded RNA templates. 5 × gDNA wiper Mix can completely remove the genomic contaimination in the RNA template, so there is no need to design intron-spanning qPCR primers. 4 × HiScript IV qRT SuperMix contains all the components required for the reverse transcription, just add template RNA and RNase-free ddH2O to start the reaction.

Components

	Components	*NB-54-0395-01*100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	5 × gDNA wiper Mix	300 μl
■	4 × HiScript IV qRT SuperMix[a]	500 μl
■	4 × No RT Control Mix[b]	50 μl

a. It contains HiScript IV Reverse Transcriptase、RNase inhibitor、dNTP Mix、Random primer/Oligo (dT)20VN primer mix, etc.

b. Except for HiScript IV Reverse Transcriptase, the remaining components are consistent with the 4 × HiScript IV qRT SuperMix, which is used to prepare No RT control reaction system.

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for reverse transcription reactions of animal, plant and microbial RNA. The obtained cDNA 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 or ice box.
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RNA

- High quality RNA is essential for obtaining high quality cDNA. Please verify the RNA integrity by electrophoresis before the experiment.

qPCR Reagent Selection Guide

- AceQ Universal Probe Master Mix V2 (Neo Biotech #NB-54-0172) or Taq Pro Universal SYBR qPCR Master Mix (Neo Biotech #NB- 54-0226) can be selected as the qPCR reagent.

Notes

For research use only. Not for use in diagnostic procedures.

1. 5 × gDNA wiper Mix, 4 × HiScript IV qRT SuperMix and 4 × No RT Control Mix contain high concentration of glycerol. Please centrifuge briefly, pipette up and down to mix thoroughly before use and pipette accurately.
2. It is recommended to add no more than 1 µg of total RNA to a 20 µl reverse transcription reaction system. If target genes with low expression levels, the amount of total RNA can be up to 5 µg. Excess RNA will cause CT values to deviate from the linear range in qPCR assays.
3. The obtained cDNA is only applicable for qPCR, not for PCR amplification of long fragments used for cloning and other downstream experiments. If necessary, Neo Biotech Universal RT-PCR/RT-qPCR Mix series is recommended.
4. The obtained cDNA can be directly used for qPCR detection. The volume of undiluted cDNA template should be ≤1/10 of qPCR reaction system.
5. Genome elimination is optional and reverse transcription can be performed using 4 × HiScript IV qRT SuperMix.
6. Do not use 5 × gDNA wiper Mix with other reverse transcription reagents as they may not contain components to terminate the function of gDNA wiper, resulting in inaccurate qPCR results.

Experiment Process

1.Removal of Genomic DNA

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

Components	Volume
RNase-free ddH2O	to 15 µl
5 × gDNA wiper Mix	3 µl
Template RNA	Total RNA: 1 pg - 1 µg

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

2.Preparation of reverse transcription reaction mixture

Add 4 × HiScript IV qRT SuperMix to the mixture of previous step:

Components	Volume
4 × HiScript IV qRT SuperMix	5 µl
Mixture from Step 1	15 µl

Gently pipette up and down several times to mix thoroughly.

No RT Control Reaction (Optional)

No RT Control Reaction is a negative control which contains no Reverse Transcriptase and is used to indicate whether there is residualgenomic DNA in RNA template.

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

Components	Volume
4 × HiScript IV qRT SuperMix	5 µl
Mixture from Step 1	15 µl

Gently pipette up and down several times to mix thoroughly.

3.Reaction Program

Temperature	Time
37℃*	15min
85℃	5 sec

* For template with complex secondary structures or high GC content, the temperature can be increased to 50°C, which will benefit the yield.

The product can be used for qPCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage. Avoid repeated freeze-thaw cycles of the cDNA.