# HiScript III All-in-one RT SuperMix Perfect for qPCR

**R333**

Version 22.1

## Product Description

HiScript III All-in-one RT SuperMix Perfect for qPCR is an upgraded version of HiScript III RT SuperMix for qPCR (+gDNA wiper). Within one step, genomic removal and reverse transcription reactions can be achieved simultaneously. The simpler and faster opereation ensure reduce risk of sample contamination and RNA degradation. This product contains HiScript III Reverse Transcriptase, heat-labile DNase and an optimized buffer system. The heat-labile DNase works quickly and efficiently; and it could be inactivated easily. The RT product is compatible with dye-based and probe-based qPCR, and can perform high-performance gene expression analysis.

## Components

| Components | R333-01 100 rxns (20 μl/rxn) |
|---|---|
| RNase-free $ddH_2O$ | 2 × 1 ml |
| Enzyme Mix* | 100 μl |
| 5 × All-in-one qRT SuperMix* | 400 μl |
| No RT Control Mix* | 20 μl |

* Please keep Enzyme Mix, 5 × All-in-one qRT SuperMix and No RT Control Mix on ice to keep the activity of heat-labile DNase.

## 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

**qPCR Reagents Selection Guide**

Dye-based qPCR reagent (e.g., Taq Pro Universal SYBR qPCR Master Mix, Vazyme #Q712) or probe-based qPCR reagent (e.g., Taq Pro Multiple Probe qPCR Mix, Vazyme #QN213-EN).

## Notes

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

1. After the preparation of reaction system, please gently pipette up and down 8 - 10 times to mix thoroughly. Otherwise, the stability of the experimental data will be affected.
2. Please keep Enzyme Mix, 5 × All-in-one qRT SuperMix and No RT Control Mix on ice to keep the activity of heat-labile DNase.
3. Enzyme Mix, 5 × All-in-one qRT SuperMix and No RT Control Mix contain high concentration of glycerol, please centrifuge briefly before use and pipette up and down to mix thoroughly.
4. 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.
5. If the stock solution of cDNA product is directly used as the template of qPCR, it is recommended that the volume of cDNA product should be ≤1/10 of the total volume of qPCR system.
6. The product can be used for qPCR immediately or be stored at -30 ~ -15℃ for 6 months. It is recommended to store in aliquots at -85 ~ -65℃ for long term storage. cDNA should avoid repeated freezing and thawing.

## Experiment Process

### 1. Reaction System

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

| | | |
|---|---|---|
| RNase-free dd$H_2O$ | to 20 μl | □ |
| 5 × All-in-one qRT SuperMix | 4 μl | ■ |
| Enzyme Mix | 1 μl | ■ |
| Template RNA | Total RNA: 1 pg - 1 μg | |

Gently pipette up and down 8 - 10 times to mix thoroughly*, then centrifuge it briefly to the bottom of the tube.

* Please be sure to mix thoroughly; otherwise, the stability of the experimental data will be affected.

**No RT Control reaction (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 dd$H_2O$ | to 20 μl | □ |
| 5 × All-in-one qRT SuperMix | 4 μl | ■ |
| No RT Control Mix | 1 μl | ■ |
| Template RNA | Total RNA: 1 pg - 1 μg | |

Gently pipette up and down 8 - 10 times to mix thoroughly*, then centrifuge it briefly to the bottom of the tube.

* Please be sure to mix thoroughly; otherwise, the stability of the experimental data will be affected.

### 2. Reaction Program

| | |
|---|---|
| 50℃ | 15 min |
| 85℃ | 5 sec |

The product can be used for qPCR immediately or be stored at -30 ~ -15℃ for 6 months. It is recommended to store in aliquots at -85 ~ -65℃ for long term storage. cDNA should avoid repeated freezing and thawing.