# HiScript III RT SuperMix for qPCR (+g DNA wiper)

**NB-54-0183**

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## Introduction

Hiscript® III RT SuperMix for qPCR (+gDNA wiper) is an upgraded version of Hiscript® II RT SuperMix for qPCR (+gDNA wiper), including a new generation of reverse transcriptase Hiscript® III Reverse Transcriptase and optimized Buffer. This SuperMix further improves the efficiency of cDNA synthesis, and is suitable for two-step qRT-PCR detection. The 4 x gDNA wiper Mix in the kit completely removes residual genomic DNA from the RNA template, ensuring more reliable quantitative results and simplifying qPCR primer design without the need to design primers across introns; 5 x Hiscript® III QRT SuperMix contains all components required for the reverse transcription reaction can be rapidly started by adding template RNA and water, and the gDNA wiper is terminated to ensure the integrity of the cDNA. The product yield is compatible to dye method qPCR and probe qPCR for high performance gene expression analysis.

## Package Information

| Components | **NB-54-0183rxn (20μl/rxn)** |
|---|---|
| RNase free dd$H_2O$ | 2x 1 ml |
| 4× gDNA wiper Mix | 400 μl |
| 5x Hiscript III qRT SuperMix[a] | 400 μl |
| 5x No RT Control Mix[b] | 40 μl |

a. Contains Buffer、dNTP、 Hiscript III Reverse Transcriptase、 Rnase inhibitor、 Random primers,/Oligo$(dT)_{20}$VN primer mix.
b. Contains the same components with 5× Hiscript III QRT SuperMix except Hiscript III Reverse Transcriptase.

## Storage

Store at -30℃ to -15℃. Transportation condition is -20℃ to 0℃.

## Unit Definition

1. All components have been tested to be free of exonuclease, exonuclease and RNase.

**Functional Detection** : Reverse transcription reaction was performed using total RNA of 1 μg Hela cells as a template, and the expression levels of four genes were detected by qPCR, and the CT value was similar among the products in the batches. 100 ng of human genomic DNA was mixed into total RNA of 1 μg of Hela cells, and after treatment with gDNA wiper Mix, qRT-PCR of two pairs of control primers was performed. The CT value of No Rt Control >40.

## Experimental Preparation and Guidelines

Materials Needed:

- 1.5 ml Rnase-free centrifuge tube, 0.2 ml PCR tube, pipette tip
- Pipette, PCR instrument, ice or moving ice box

RNA

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

qPCR Reagent Selection Guide:

- The first strand cDNA product can be used directly as a template for the qPCR reaction. The volume of the cDNA product recommended asa template does not exceed 1/10 of the qPCR reaction volume.

## Protocol

1. Remove genomic DNA

Prepare the following mixture in an RNase-free centrifuge tube:

| | |
|---|---|
| RNase free $ddH_2O$ | to 16 μl |
| 4× gDNA wiper Mix | 4 μl |
| Template RNA | Total RNA:1 pg - 1 μg |

Mix gently with a pipette. Incubate at 42℃ for 2 min.

2. Preparation of Reverse Transcription Reaction Mixture

Add 5x Hiscript III Enzyme Mix to the mixture of previous step

| | |
|---|---|
| 5x Hiscript III qRT SuperMix | 4 μl |
| Mixture of previous step | 16 μl |

Mix gently with a pipette.

No RT Control Reaction (Optional)

No RT Control Reaction is a negative control reaction without reverse transcriptase to test DNA residues in the RNA template.
Prepare the following mixture in an RNase-free centrifuge tube

| | |
|---|---|
| 5x No RT Control Mix | 4 μl |
| Mixture of the first step | 16 μl |

Mix gently with a pipette.

3. Perform the reverse transcription reaction under the following conditions:

| | |
|---|---|
| 37℃ * | 15 min |
| 85℃ | 5 sec |

* For template with complicated secondary structures or high GC content, you can raise the reaction temperature to 50℃ to increase the cDNA yield.

The product can be directly used in qPCR reactions, or store at -20℃ and used within six months. It is recommended to aliquots and store at -80℃ for a long-term storage. Avoid repeated freezing and thawing.

## Notes

1. 4 x gDNA wiper Mix, 5 x Complete III qRT SuperMix and 5 x No RT Control Mix are containing high concentrations of glycerol, centrifuged briefly and mix well by pipetting gently before use.
2. It is recommended that adding no more than 1 μg of total RNA on 20 μl reverse transcription reaction. If the target genes with low mRNA expression level, add up to 5 μg of Total RNA, or joining the RNA content is too high, may exceed the linear range of the subsequent quantitative PCR.
3. The cDNA product is only suitable for qPCR reactions and is not suitable for long-fragment PCR amplification of downstream experiments such as cloning
4. Reverse transcription can be performed directly with 5 x Hiscript III QRT SuperMix without the genome removal step, but do not use 4 x gDNA wiper Mix with reagents without genome removal module, as the Mix in the kits without genome removal module contains no components that terminate the gDNA wiper reaction and may affect subsequent qPCR results