# HyperScript™ RT SuperMix for qPCR (with gDNA wiper)

## Introduction

HyperScript™ RT SuperMix for qPCR (with gDNA wiper) is the premixed solution for reverse transcription reaction, based on HyperScript™ Reverse Transcriptase (Cat. No. K1071**).** HyperScript™ Reverse Transcriptase is a new enzyme obtained through genetic engineering based on M-MLV (RNase $H^-$) Reverse Transcriptase. In comparison, HyperScript™ Reverse Transcriptase reduces RNase H activity and increases thermal stability. HyperScript™ Reverse Transcriptase can withstand higher reaction temperatures and is suitable for reverse transcription of RNA templates with complex secondary structures.

HyperScript™ RT SuperMix for qPCR (with gDNA wiper) is suitable for two-step qRT-PCR detection ,5×SuperMix contains all the components required for reverse transcription, and the reaction can be carried out quickly by only adding template RNA and RNase Free dd$H_2O$.

The kit is very suitable for the reverse transcription reaction of the low concentration RNA template. 5×SuperMix will not freeze at -20°C, easy to use. The 4× gDNA wiper included in the product quickly and thoroughly removes potential genomic interference from the system before reversing transcription. This product is specially optimized for qPCR. The proportionally optimized Oligo $(dt)_{23}$vn primer mix/random primers enables cDNA synthesis to start from various regions of the RNA transcript with the same reverse transcription efficiency, maximizing the authenticity and repeatability of the qPCR results. Reverse transcription products are suitable for qPCR by SYBR Green and probe methods. The corresponding reagents can be selected for high performance gene expression analysis according to the experimental purpose.

## Components and Storage

| Components | 50 rxns (20 μL reaction) | 100 rxns (20 μL reaction) |
|---|---|---|
| RNase Free dd$H_2O$ | 1 mL | 2×1 mL |
| 4×gDNA wiper mix | 200 μL | 400 μL |
| 5× RT SuperMix | 200 μL | 400 μL |
| 5× No RT control Mix | 20 μL | 40 μL |
| Store the components at -20°C. | | |

## Protocol

### 1. Remove genomic DNA：prepare the following mixed solution in RNase free PCR tube.

| Components | volume |
|---|---|
| RNase Free ddH2O | Up to 16 uL |
| 4×gDNA wiper mix | 4 uL |
| Templet RNA | Total RNA: 1 pg -1 μg |

Gently mix the above reactions and perform incubation operations.

| temperature | time |
|---|---|
| 37°C | 2 min |
| 55°C | 5 min |

### 2. Preparation of first chain cDNA synthetic reaction in RNase free PCR tube.

| Components | volume |
|---|---|
| 5×SuperMix | 4 μL |
| Reaction solution of Step 1 | 16 μL |

**No RT Control reactions (optional)**

No RT Control refers to the negative reverse transcription reaction without HyperScript™ Reverse Transcriptase, which is used to test whether there are genomic DNA residues in the RNA template.

prepare the following mixed solution in RNase free PCR tube.

| Components | volume |
|---|---|
| 5×No RT control Mix | 4 μL |
| Reaction solution of Step 1 | 16 μL |

### 3. Reverse transcription reaction

Mix the above reactants gently, centrifuge instantly and set the reverse transcription procedure according to the following table:

| temperature | time |
|---|---|
| 25°C | 2 mins |
| 42-50°C | 15 mins |
| 95°C | 1 min |

**Note:** HyperScript™ Reverse Transcriptase still has good amplification ability for RNA templates with complex secondary structures. For RNA templates that need to be validated by qPCR in the next step, it is usually

recommended to perform the reaction at 50°C.

The obtained reverse transcript products can be used immediately for qPCR reactions and can also be stored in -20°C for short-term preservation and -80°C for long-term preservation, avoiding repeated freezing and thawing. Storage at -20°C should not exceed 1 week, and it is generally recommended to keep it at -80°C.

## Matters need attention

1. Experiments were operated on ice to avoid RNase contamination during the process.
2. The purity of RNA may affect the amount of cDNA synthesis, so the RNA extraction process should pay attention to prevent RNA degradation.
3. The 5×SuperMix and 5×SuperMixNo RT Control Mix contain high concentration of glycerin, before using please briefly centrifuge collection to the bottom of the reaction tube and mix it gently and thoroughly with the liquid remover.
4. For the 20μl reverse transcription reaction system, it is recommended to add no more than 1μg Total RNA, if the target gene expression is very low, add up to 5μg total RNA, otherwise the amount of addition is too high, which may be beyond the linear range of subsequent qPCR.
5. Make sure RNA is soluble in water rather than in TE if the volume of added template is too much (more than 2μl), as the EDTA in the TE will inhibit the reverse transcription reaction.
6. The RNA template may be contaminated by genomic DNA if the CT value difference between No RT Control and experimental group is less than 5.
7. The cDNA products are only suitable for qPCR reactions and not for long fragment PCR amplification of downstream experiments such as cloning. First-Strand cDNA Synthesis Kit (Cat. No. K1072)/ First-Strand cDNA Synthesis SuperMix (Cat. No. K1073)/First-Strand cDNA Synthesis SuperMix (with gDNA wiper) (Cat. No. K1173) are recommended if required.

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