# HiScript® III 1 st Strand cDNA Synthesis Kit (+ gDNA wiper)

**R312-01**

Version 8.1

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## Introduction

Hiscript III 1 st Strand cDNA Synthesis Kit (+ gDNA wiper) is an upgraded version of Hiscript II 1st Strand cDNA Synthesis Kit (+ gDNA wiper) containing a new generation of reverse transcriptase Hiscript III Reverse Transcriptase and Buffer optimized for reverse transcription. This product improves the efficiency of first strand cDNA synthesis. The 5× gDNA wiper Mix in the kit can quickly remove genomic DNA contamination at 42℃ for 2 min, which makes the results more reliable and simplifies qPCR primer design process without the need to design primers across introns. The kit contains two separated primers: the reverse transcription primers Oligo $(dT)_{20}VN$ and Random hexamers, allowing users to choose primers flexibly for subsequent experiments as needed. The kit can synthesize full-length cDNA (up to 20 kb) for downstream experiments such as cloning, and can also synthesize a highly uniform cDNA for qPCR quantification.

## Package Information

| Components | R312-01 100 rxn (20 μl/rxn) | R312-02 100 rxn (20 μl/rxn) |
|---|---|---|
| RNase free $ddH_2O$ | 1 ml | 1 ml |
| 5× gDNA wiper Mix | 100 μl | 200 μl |
| 10x RT Mix[a] | 100 μl | 200 μl |
| Hiscript III Enzyme Mix[b] | 100 μl | 200 μl |
| Oligo $(dT)_{20}VN$ | 50 μl | 100 μl |
| Random Hexamers | 50 μl | 100 μl |

a. Contains dNTP

b. Contains RNase inhibitor

## Storage

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

10× RT Mix contains a high concentration of DTT, and there may be precipitation at low temperatures. Restore to room temperature before use and gently shake and mix until the precipitate is redissolved.

## Quality Control

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

**Functional detection 1**: 100 ng human genomic DNA was mixed into the total RNA of 1 ug HeLa cells. After treatment with gDNA wiper Mix, perform qRT-PCR with two pairs of quality control primers, and the Cr value of No RT Control was > 40.

**Functional detection 2**: Reverse transcription was performed using total RNA of 1 ug HeLa cells as template. Three DNA fragments of different lengths (4.8 Kb, 7.1 Kb, 15 Kb) were amplified by PCR. The target band was single and its brightness was similar in batches of products.

**Functional Detection 3**: Reverse transcription was performed using total RNA of 1 ug HeLa cells as template. qPCR was performed to detect the expression of four genes. The value of Cr was similar in batches of products.

## Notes

### Prevent RNase contamination

Keep the experiment area clean; Wear clean gloves and masks, and use new centrifuge tubes, tips and other supplies to ensure experiment is RNase free.

### Choosing Primers for PCR

- Oligo $(dT)_{20}$ VN hybridizes at high efficiency to the 3´ poly(A) region found in most mature eukaryotic mRNA. It is the first choice for most cases and generally results in a high yield of the full-length cDNA.

- Gene specific primers (GSP) have the highest specificity. But under some circumstances, GSP used for PCR reaction cannot effectively synthesize cDNA. Select Oligo $(dT)_{20}VN$ or Random Hexamers in this case.
- Random Hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be templates of Random Hexamers. When the target area of RNA has acomplex secondary structure or is GC-rich, and Oligo dT or gene specific primers (GSP) cannot effectively synthesize cDNA, select Random Hexamers.

**Choosing Primers for qPCR**

- Oligo dT mixed with Random Hexamers can lead to cDNA synthesis at equivalent efficiency and helps to improve the reproducibility of quantitative results.

## Protocol

### ◇ Guidelines for PCR reaction

**1. Denaturation of RNA template***

Set up the following mixture in an RNase-free centrifuge tube.

| | |
|---|---|
| RNase free $ddH_2O$ | to 8 μl |
| Total RNA | 10 pg - 5 μg |
| Or Poly (A)+ RNA | 10 pg - 500 ng |

Incubate at 65˚C for 5 minutes, place on ice rapidly and let rest for 2 minutes.

*The denaturation step helps to open the secondary structures to improve the first strand cDNA yield. For cDNA fragment longer than 3 kb, do not omit the denaturation step.

**2. Remove genomic DNA**

Set up the following mixture in an RNase-free centrifuge tube.

| | |
|---|---|
| Mixture of previous step | 8 μl |
| 5× gDNA wiper Mix | 2 μl |

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

**3. Preparation of a first strand cDNA synthesis reaction mixture**

| | |
|---|---|
| Mixture of previous step | 10 μl |
| 10x RT Mix | 2 μl |
| Hiscript III Enzyme Mix | 2 μl |
| Oligo $(dT)_{20}VN$ | 1 μl |
| Or Random Hexamers | |
| RNase-free $ddH_2O$ | 5 μl |

Mix gently with a pipette.

*This product is also suitable for reverse transcription using gene-specific primers (GSP). To avoid the potential effect of gDNA wiper on GSP, please add gene-specific primers (2 pmol) to the mixure.

**4. Perform the first strand cDNA synthesis reaction under the following conditions:**

| | |
|---|---|
| 25˚C[a] | 5 min |
| 37˚C[b] | 45 min |
| 85˚C | 5 sec |

a. This step is required only when using the Random hexamers. Please omit this step when using Oligo (dT) 20VN or Gene Specific Primer.

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

The product can directly be used in PCR reactions, or store at -20˚C and used within six months. It is recommended to aliquots and store at -70˚C for a long-term storage. Avoid repeated freezing and thawing.

## ◇ Guidelines for qPCR reaction

### 1. Remove genomic DNA

Set up the following mixture in an RNase-free centrifuge tube:

| | |
|---|---|
| RNase free $ddH_2O$ | to 10 μl |
| 5× gDNA wiper Mix | 2 μl |
| Total RNA | 10 pg - 1 μg |
| Or Poly (A)+ RNA | 10 pg - 100 ng |

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

### 2. Preparation of a first strand cDNA synthesis reaction mixture

Set up the following mixture in an RNase-free centrifuge tube:

| | |
|---|---|
| Mixture of previous step | 10 μl |
| 10x RT Mix | 2 μl |
| Hiscript III Enzyme Mix | 2 μl |
| Oligo $(dT)_{20}VN$ | 1 μl |
| Random Hexamers | 1 μl |
| RNase-free $ddH_2O$ | 4 μl |

Mix gently with a pipette.

*This product is also suitable for reverse transcription using gene-specific primers (GSP). To avoid the potential effect of gDNA wiper on GSP, please add gene-specific primers (2 pmol) to the mixure.

### 3. Perform the first strand cDNA synthesis reaction under the following conditions:

| | |
|---|---|
| 37˚C* | 15 min |
| 85˚C | 5 sec |

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

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

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