# HiScript II 1st Strand cDNA Synthesis Kit

**R211**

Version 22.2

## Product Description

HiScript II Reverse Transcriptase is a novel reverse transcriptase obtained by *in vitro* molecular evolution technology based on M-MLV (RNase H-) Reverse Transcriptase. Compared with HiScript Reverse Transcriptase, HiScript II further improves the thermal stability and is suitable for reverse transcription of RNA templates with complex secondary structures. In addition, HiScript II adds several point mutations that further enhances template binding affinity and cDNA synthesis efficiency, and has higher tolerance to common reverse transcription inhibitors.

This product is 1st strand cDNA synthesis kit based on HiScript II Reverse Transcriptase. It contains all the components necessary for high quality first strand cDNA synthesis, and the product is suitable for subsequent PCR, qPCR and other experiments. The 2 × RT Mix contains an optimized buffer and dNTPs. The HiScript II Enzyme Mix contains the HiScript II Reverse Transcriptase and the RNase inhibitor. Oligo $(dT)_{23}VN$ has stronger anchoring ability for poly $A^+$ mRNA than Oligo $(dT)_{18}$, which makes reverse transcription more efficient. Oligo $(dT)_{23}VN$, Random primers or Gene Specific Primers (GSP) can be selected as reverse transcription primers according to requirements. The kit can be used to synthesize full-length cDNA (up to 20 kb) for cloning and other downstream experiments, as well as highly uniform cDNA for qPCR.

## Components

| Components | R211-01 50 rxns (20 μl/rxn) | R211-02 100 rxns (20 μl/rxn) |
|---|---|---|
| RNase-free $ddH_2O$ | 1 ml | 1 ml |
| 2 × RT Mix[a] | 500 μl | 1 ml |
| HiScript II Enzyme Mix[b] | 100 μl | 200 μl |
| Oligo $(dT)_{23}VN$ (50 μM) | 50 μl | 100 μl |
| Random hexamers (50 ng/μl) | 50 μl | 100 μl |

a. It contains 1 mM each dNTP.

b. It contains RNase inhibitor.

## Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

## Applications

It is applicable for reverse transcription of animal, plant and microbial RNA.

## Notes

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

**Primers selected**

**For PCR**

- For eukaryotic RNA templates, use Oligo dT primer to obtain the highest yield of full-length cDNA by pairing with 3' Poly A of eukaryotic mRNA.
- GSP has the highest specificity. If GSP fails in the 1st strand cDNA synthesis, Oligo $(dT)_{23}VN$ or Random hexamers can be used for reverse transcription.
- Random hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be used as the template of Random hexamers. Random hexamers can be used as primers, when Oligo $(dT)_{23}VN$ or GSP can not effectively guide cDNA synthesis for the target region has complex secondary structure and high GC content, or the template is prokaryotic origin.

**For qPCR**

- Mixing Oligo $(dT)_{23}VN$ with Random hexamers enables the same efficiency of cDNA synthesis in each region of the mRNA, which helps to improve the authenticity and reproducibility of quantitative results.

## Experiment Process

### ◇ For PCR

**1. RNA Denaturation***

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

| | | |
|---|---|---|
| RNase-free dd$H_2O$ | to 8 μl | □ |
| Oligo $(dT)_{23}VN$ (50 μM) | | ■ |
| or Random hexamers (50 ng/μl) | 1 μl | ■ |
| or Gene Specific Primers (2 μM) | | |
| Total RNA | 1 pg - 5 μg | |
| or Poly $A^+$ RNA | 10 pg - 500 ng | |

Incubate at 65℃ for 5 min and then chill on ice immediately for 2 min.

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

**2. Preparation of reaction solution for 1st strand cDNA synthesis**

| | | |
|---|---|---|
| Mixture of Step 1 | 8 μl | |
| 2 × RT Mix | 10 μl | ■ |
| HiScript II Enzyme Mix | 2 μl | ■ |

Gently pipette up and down several times to mix thoroughly.

**3. Reaction Program**

| | |
|---|---|
| 25℃[a] | 5 min |
| 50℃[b] | 45 min |
| 85℃ | 2 min |

a. Only necessary when using Random hexamers. Please skip this step when using Oligo $(dT)_{23}VN$ or GSP.

b. For templates with complicated secondary structure or high GC content, the temperature can be increased to 55℃, which will benefit the yield.

The product can be used for PCR immediately or be stored at -20℃ for 6 months. It is recommended to store in aliquots at -70℃ for long term storage. cDNA should avoid repeated freezing and thawing.

### ◇ For qPCR

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

| | | |
|---|---|---|
| RNase-free dd$H_2O$ | to 20 μl | □ |
| 2 × RT Mix | 10 μl | ■ |
| HiScript II Enzyme Mix | 2 μl | ■ |
| Oligo $(dT)_{23}VN$ (50 μM) | 1 μl | ■ |
| Random hexamers (50 ng/μl) | 1 μl | ■ |
| Total RNA | 1 pg - 1 μg | |
| or Poly $A^+$ RNA | 10 pg - 100 ng | |

Gently pipette up and down several times to mix thoroughly.

**2. Reaction Program**

| | |
|---|---|
| 25℃ | 5 min |
| 50℃* | 15 min |
| 85℃ | 2 min |

* For templates with complicated secondary structure or high GC content, the temperature can be increased to 55℃, which will benefit the yield.

The product can be used for qPCR immediately or be stored at -20℃ for 6 months. It is recommended to store in aliquots at -70℃ for long term storage. cDNA should avoid repeated freezing and thawing.