HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper)

R212

Version 22.1

Product Description

HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper) is a dedicated kit for 1st strand cDNA synthesis that includes the removal step of genomic DNA. Compared with the previous generation of HiScript Reverse Transcriptase, the HiScript II Reverse Transcriptase in this kit has greatly improved thermal stability, template binding affinity and inhibitor tolerance.

The gDNA wiper used in this kit can quickly remove genomic DNA contamination within 2 min, ensuring more reliable subsequent results and simplifying the design of qPCR primers without the need to span an exon-exon junction. 10 × RT Mix contains optimized buffer system and dNTP; HiScript II Enzyme Mix contains HiScript II Reverse Transcriptase and RNase inhibitor. The Oligo $(dT)_{23}VN$ included in this product has the stronger anchoring ability of Poly A^+ RNA than Oligo $(dT)_{18}$, making 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. With flexible and optional subsequent steps, the kit can be used to synthesize full-length cDNA (up to 20 kb) for cloning or efficiently synthesize the highly uniform cDNA for qPCR.

Components

	Components	R212-01 50 rxns (20 μl/rxn)	R212-02 100 rxns(20 μl/rxn)
☐	RNase-free ddH_2O	1 ml	1 ml
■	4 × gDNA wiper Mix	200 μl	400 μl
■	10 × RT Mix[a]	120 μl	240 μl
■	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 dNTP.

b. It contains RNase inhibitor.

Storage

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

10 × RT Mix contains high concentration of DTT, which may precipitate at low temperature. Please restore to room temperature, shake gently and mix thoroughly, wait to redissolve the precipitation before use.

Applications

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

Notes

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

Prevent RNase contamination

Please keep the experiment area clean; wear disposable gloves and masks; use RNase-free consumables such as centrifuge tubes and pipette tips.

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 in a recommended ratio enables the same efficiency of cDNA synthesis in each region of the mRNA, which helps to improve the authenticity and reproducibility of quantitative results.
- Reverse transcription can be performed directly without the genomic removal step, and the reaction volume can be supplemented with RNase-free ddH_2O. The results obtained will be the same as using the HiScript II 1st Strand cDNA Synthesis Kit (Vazyme #R211).

Experiment Process

◇ **For PCR**

1. RNA Denaturation*

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

RNase-free ddH_2O	to 12 μl	□
Oligo $(dT)_{23}VN$ (50 μM)		■
or Random hexamers (50 ng/μl)	1 μl	■
Total RNA	10 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 fragments longer than 3 kb, please do not ignore the denaturation step.

2. Removal of genomic DNA

Mixture of Step 1	12 μl	
4 × gDNA wiper Mix	4 μl	■

Gently pipette up and down several times to mix thoroughly. Incubate at 42℃ for 2 min.

3. Preparation of reaction solution for 1st strand cDNA synthesis

Mixture of Step 2	16 μl	
10 × RT Mix	2 μl	■
HiScript II Enzyme Mix	2 μl	■

Gently pipette up and down several times to mix thoroughly.

* This product is also suitable for reverse transcription initiated by GSP. To avoid the potential impact of gDNA wiper on GSP, please add GSP (2 pmol) to the system in this step.

4. 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 complex secondary structure or high GC content, the reaction 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. Removal of genomic DNA

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

RNase-free ddH_2O	to 16 μl	□
4 × gDNA wiper Mix	4 μl	■
Oligo $(dT)_{23}VN$ (50 μM)	1 μl	■
Random hexamers (50 ng/μl)	1 μl	■
Total RNA	10 pg - 1 μg	
or Poly A^+ RNA	10 pg - 100 ng	

Gently pipette up and down several times to mix thoroughly. Incubate at 42℃ for 2 min.

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

Mixture of Step 1	16 μl	
10 × RT Mix	2 μl	■
HiScript II Enzyme Mix	2 μl	■

Gently pipette up and down several times to mix thoroughly.

* This product is also suitable for reverse transcription initiated by GSP. To avoid the potential impact of gDNA wiper on GSP, please add GSP (2 pmol) to the system in this step.

3. Reaction Program

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.